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DUBOS' SOLID AGAR MEDIUM IN ROUTINE LABORATORY DIAGNOSIS OF TUBERCULOSIS†

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Since the development of Dubos' solid agar medium for the growth of *M. tuberculosis* many laboratories have tried to fit this medium into their diagnostic program. Goldie[1] using the liquid medium and Foley[2] using both the liquid and solid media have reported favorably. Many, however, have not had success, either because disadvantageous modifications were used or because overgrowth with mold contaminants (particularly in the study of sputa and gastric specimens) tended to obscure the picture.

The present study was designed to compare the results obtained when Dubos' solid agar medium is used for the isolation of tubercle bacilli from routine specimens with the results obtained by the use of two other accepted methods—Lowenstein's modified egg medium culture and guinea pig inoculation.

Materials and Methods

The number and character of the samples received in the laboratory over a five-week period in the summer of 1949 and a ten-week period in the summer of 1950 included in the present study are shown in Table I.

Gastric aspirates were not examined microscopically for acid-fast bacilli, but the remainder were so examined. Two specimens, a sputum and a piece of ulcer tissue, were found to be positive by the Ziehl-Neelsen staining technique. The ulcer tissue did not produce typical colonies on either medium or typical lesions in the guinea pig.

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† First Award ASMT Convention, Boston, Mass., June, 1951.

Routine samples of gastric aspirates, urines, sputa, chest fluids, etc., which were sent to the laboratory were concentrated according to the procedures recommended by the United States Public Health Service.[3] Concentration was accomplished by using 4 percent NaOH and neutralizing with 2N HCL.

The preparation of Lowenstein's medium followed, in general, the directions issued by the United States Public Health Service[3] except that one liter of egg yolk was substituted for one liter of whole egg.

It seems advisable to give below the detailed procedure followed in preparing Dubos' solid medium[4] because there have been modifications since some of the earlier trials were made. No great difficulty was encountered in making up the medium when directions were followed with great care.

DUBOS' OLEIC ACID ALBUMIN MEDIUM

1. Basal Medium:

KH_2PO_4	1.0 gm.
$NaHPO_4 \cdot 12\ H_2O$	6.0 gm.
Asparagine	1.0 gm.

Heat in 100 cc. distilled water to dissolve. Add:

N-Z Amine, Type B (Sheffield Farms)	1.0 gm.
**Ferric Ammonium Citrate (brown)	0.05 gm. (5 cc. of 1% soln.)
*$MgSO_4 \cdot 7\ H_2O$	0.01 gm. (1 cc. of 1% soln.)
*$CaCl_2$	0.0005 gm. (1 cc. of 0.05% soln.)
*$ZnSO_4$	0.0001 gm. (1 cc. of 0.01% soln.)
*$CuSO_4$	0.0001 gm. (1 cc. of 0.01% soln.)

Bring up to 1000 cc. with distilled water. Adjust to pH to 6.5 using concentrated HCL (about 1 cc. per liter of medium).

2. Oleic Acid Albumin: To make 100 cc.

(1) Dissolve 0.12 cc. of oleic acid (0.1 gm.) (take top layer of bottle) in 10 cc. N/20 NaOH by shaking with a rotary motion in a small flask.

(2) Add 5 cc. of this solution to 95 cc. of a *neutral 5% solution* of Bovine Albumin Fraction V in .85% saline. (Albumin prepared from dehydrated Bovine Albumin Fraction V, Armour.)

(3) Sterilize by filtration through a bacteriological filter.

3. Solid Agar Oleic Acid Albumin Medium:

Add 1.5% agar to the basal medium (1) and autoclave at 15-20 lbs. for 20 minutes. Cool to 50° C. Add the oleic acid albumen (100 cc.) to 1000 cc. of basal medium. Add 25 units of penicillin per cc. of agar medium. Pour 20 cc. per standard Petri plate.

One-eighth of the concentrated specimen was placed on the surface of each of two Dubos' agar plates and was spread on the surface with a platinum wire loop. The plates were sealed with adhesive tape to prevent drying. In the last half of the 1949 series, two bottom plates were sealed face to face with adhesive tape, but this was abandoned in the subsequent work in favor

** Make up fresh for each new lot of medium.
* May be made up as stock solution and stored in ice box.

of sealing the tops to the bottoms. The reason for discarding the method of sealing bottoms together, which conserved moisture better over long periods, was that contamination was more frequent.

Approximately one-eighth of each concentrated specimen was inoculated on each of two slants of Lowenstein's medium using a Pasteur pipette. The inoculum was spread over the surface, cotton stoppers replaced, and the tops of the tubes sealed with paraffin wax.

Approximately one-half of the total concentrated specimen was mixed with 1 cc. of sterile distilled water and the total mixture (about 1.5 cc.) was injected subcutaneously into the groin of a 200-300 gm. guinea pig. The guinea pig was autopsied at the end of six weeks.

In the 1949 series it was noted that typical colonies on Dubos' medium appeared considerably earlier than on Lowenstein's medium. The Dubos' agar plates in the 1950 series were read on the fifth day of incubation and at two-day intervals to the fifteenth day. From the fifteenth day to the thirtieth day the plates were read at five-day intervals. The tenth and final reading was made at the end of six weeks. The Lowenstein slants were read at weekly intervals

In all cases, typical colonies on either Dubos' or Lowenstein's medium were verified by smearing the colonies and staining by the Ziehl-Neelsen technique. In all cases in which the cultures were not verified by the guinea pig in the initial routine test, an emulsion of the colonies was inoculated into a second guinea pig to demonstrate virulence.

Results

Dubos' observation that the virulent *M. tuberculosis* colony has a characteristic appearance was amply confirmed in this study. The fact that the colony form is diagnostic both microscopically and macroscopically is an exceedingly useful feature of the work with this medium. Not once throughout the entire study was the *M. tuberculosis* colony confused with any of the other colonies found on this medium. Four specimens showed acid-fast saprophytes on Lowenstein's medium. It is significant that these acid-fast saprophytes did not in any way confuse the true reading of Dubos' agar plates. However, one strain of M. phlei showed colonies superficially resembling M. tuberculosis. Moreover, once the technician learns the characteristic colony form on Dubos' medium, he will rarely overlook it even if there is a considerable overgrowth of contaminants. Grossly, the discrete colonies measure from 1-6 mm. in diameter, depending on the age and degree of crowding The colonies are gray-white, translucent, the surface dull to reflected light, and the edges

are scalloped. When incubated longer than 14 days, they show a denser, opaque center and the surface becomes moderately wrinkled.

Microscopically, 80x, the colony is golden in color and the scalloped border is easily seen. Young colonies show a network of fine threads somewhat radially arranged and extending to the periphery—older colonies have a ropey appearance.

The comparative performance of Dubos' agar, Lowenstein's egg medium, and the guinea pig is shown in Figure I.

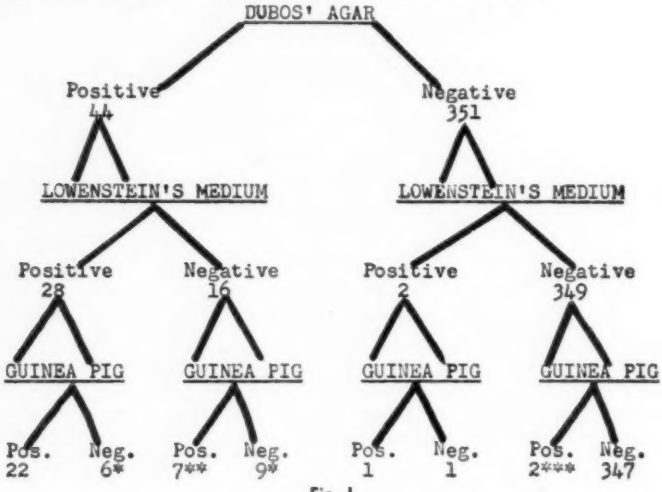

Fig. I

Figure I is constructed to include all of the specimens used in the study: showing 48 specimens in which *M. tuberculosis* was demonstrated in the initial isolation by one or more of the methods used. In the initial isolation Dubos' medium demonstrated 44, Lowenstein's medium 30, and the guinea pig 31, of the 48 specimens shown to contain *M. tuberculosis.*

The difference in the time required for the appearance of typical colonies on the Dubos' and Lowenstein's media is shown below in Figure II for the 30 positive specimens on the Dubos' agar and the 17 positive specimens on the Lowenstein's medium in the 1950 portion of the study. Lowenstein's Medium shows a definite lag. One lot of Dubos' Medium, made up with human albumin showed a much longer interval between the time of inoculation and the time of appearance of typical colonies.

* Four of these pigs died without lesions.
** In one of these specimens both tubes of Lowenstein's medium was contaminated.
*** In one of these specimens both plates of Dubos' agar were contaminated.

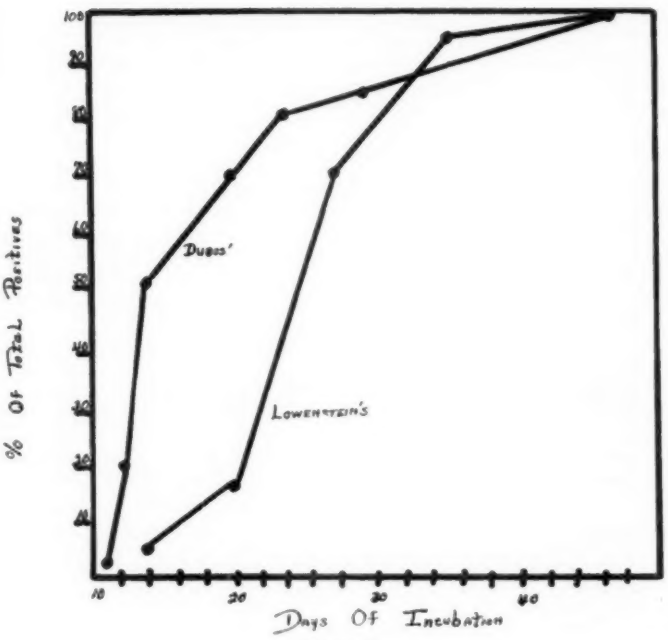

Figure II

Time of appearance of typical colonies on the two culture media.

Discussion

The fact that virulent *M. tuberculosis* has a characteristic colony type on Dubos' agar and is easily differentiated from the colonies of saprophytes or contaminants, was one of the most useful features of the medium. The concentration of penicillin (25 units per cc. in 1949 and 50 units per cc. in 1950) was nearly enough to suppress the growth of contaminants. Six percent of the tubes and plates were considered unreadable in the 1950 part of the study. According to Kirby and Dubos[5] 50-100 units of penicillin per cc. may be used to reduce contaminants without inhibiting the growth of *M. tuberculosis*. Dubos[6] currently recommends the use of 50 units of penicillin per cc. of medium. Dubos has also observed that sodium glutamate in a final concentration of 0.2 percent appears to increase the rate of growth of pathogenic acid-fast organisms. Typical colony growth could be seen on Dubos' medium up to two weeks prior to its appear-

ance on Lowenstein's medium. The rapidity with which growth occurs and the ease and certainty with which these plates can be read makes Dubos' medium of real value in routine work, especially in conjunction with other methods if two or more methods can be used.

Conclusions

In this series Dubos' solid agar gave results which compared very favorably with those obtained by the use of modified Lowenstein's medium or the guinea pig inoculations when applied to the isolation of *M. tuberculosis* from routine laboratory specimens. Positive results were obtained earlier. Contamination did not offer an insuperable problem. The colony type is diagnostic.

Table I

NUMBER AND SOURCE OF SPECIMENS INCLUDED IN THE STUDY

Source	1949	1950	Totals
Gastric Aspirates	90	110	200
Urines	43	56	99
Sputa	12	34	46
Pleural Fluids	15	18	33
Bronchial Fluids	2	5	7
Tissues	1	3	4
Spinal Fluids	3	1	4
Vaginal Discharges	..	1	1
Synovial Fluids	1	..	1
Totals	167	228	395

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Miss Alice Tracey and Miss Rebekah DuBois, Department of Medicine, Cornell-New York Hospital, New York, for detailed information on preparation and use of Dubos' solid agar medium.

RECENT ADVANCES IN PARASITOLOGICAL DIAGNOSTIC TECHNICS[1]

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Unlike many other diseases and dysfunctions which can be diagnosed by a variety of laboratory tests and clinical symptoms, infections and diseases caused by parasites can only be confirmed by finding the causative organisms or their products. Only this is pathognomic. Parasitic diseases simulate in their symptomatology many other diseases. A variety of laboratory tests—blood pictures, skin tests or complement-fixation or other serological tests—can be used along with symptomatology and careful epidemiological analysis to give a presumptive diagnosis, but only by actually finding the parasites can the diagnosis be confirmed.

The object, therefore, in laboratory diagnosis of dysfunctions in which parasites are suspected, is to find the organisms.

Upon finding the actual organism depends, also, the ability to differentiate it from other closely related species which, while also parasitic, may not be pathogenic. Incorrect specific diagnosis which would entail an unnecessary course of treatment to the patient as a result is a grave error, although, perhaps, not as grave as not finding a parasite at all.

As in all fields of biological and medical sciences, great advances are being made in diagnostic procedures. There is great activity along many fronts, and over 1000 papers per year report on parasitological techniques alone. Several hundred of these can be listed as new ideas.

However, one thing is to be kept in mind—not all new ideas are advances. Each new technique must be thoroughly tried out before adoption for practicability in many laboratories, under a variety of conditions, with a variety of equipment available, by a variety of technicians, and evaluated accordingly. We must remember that we are dealing with individuals. Slight variations in procedure, difficult to detect even with careful watching, will make the results turn out well for one technician and be useless in the hands of another.

There are great advantages in using one technique which you know well. If it works for you, while, of course, fulfilling its

[1] Lecture delivered to Second State Seminar, Illinois Medical Technologists Association, Mt. Sinai Hospital, Chicago, Illinois, March 4, 1951.

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function of demonstrating the parasites when they are present in the specimen, continue to use it.

However, there is a lot to be said for standardization of techniques. Standardization is important. It cuts down the number of variables between different laboratories which results in variations in results. The widespread discrepancies in survey results now reported in the literature are disheartening and frustrating. Much of this is due to differences in techniques.

Our goal is standardization of techniques. Yet such standardization is a long way off—if we ever do achieve it. We do not desire standardization merely for the sake of regimentation or the compilation of a mass of epidemiological figures (although this last is a desirable goal), but standardization is desired so that the best available technique is used and the results can be expressed on a *quantitative* basis. The Laboratories Division of the U. S. Public Health Service's Communicable Disease Center in Atlanta, Georgia, is making a valuable contribution to this project in several ways: firstly, through their widespread teaching activities, they are training technicians from every state in the country; secondly, through their research program they have developed several new, more satisfactory techniques; and finally, they have an organized procedure testing program with most of the state Public Health Laboratories. In this, they send out standardized unknown samples containing various parasites. The technicians in each laboratory make the examinations using their own local techniques and report their results back to Atlanta. In this way, the reliability of their procedures can be judged and checked, as well as their technicians' training and reporting.

Usually, it can be said that where there is a wide variety of techniques described and used, not any one of them is really satisfactory. However, not one technique yet perfected is all inclusive; that is, will reveal all the parasites of every stage under all conditions and degrees of infection. Therefore, it is necessary to use several techniques and the technician should be acquainted with a range of different methods.

This audience has different backgrounds and training, performs different laboratory functions, and has different personal interests. The field of parasitological techniques is so diversified that it would be difficult to cover it all in one talk. Hence today I am going to restrict this talk to a variety of different select topics and mention some of the outstanding advances made in the diagnostic field during the past several years. For the most part, they will be those which have not as yet found their way into the standard laboratory handbooks or parasitology texts. The techniques will not be described in detail, but references

will be given to where the details can be found. For the most part, we will stress the general relationship of these advances to the problems at hand.

A brief review of the general parasitological diagnostic methods used in establishing the casual relationship of a dysfunction with a specific parasite may not be out of place. This, to show not only the range of techniques available but also the place of those to be discussed in the general scheme.

1. *General Procedures in Laboratory Investigation and Diagnosis of Parasitic Infections.*

Except for the somatic (tissue) parasites for which man is an accidental host and hence are caught in a cul-de-sac and can not get out, and for the ectoparasites which are already outside (external) the natural protective layers of the body, all of the internal parasites of man must themselves get out of the body or express their progeny, be they eggs, cysts, or larvae, out of the body to carry on the essential reproductive processes and to insure the continuation of the race.

To diagnose parasitic infections, therefore, we look at those *portals-of-exist* to find the progeny.

The portals-of-exit are:

1. *the feces*: utilized by parasites of the digestive tract, liver and associated organs of the alimentary canal; also to some extent by parasites of the lungs and nasopharynx. (stool or fecal examinations)

2. *mucoid secretions* from the epithelial layers of the atrial cavities of the body:

   a. *the sputum*—utilized by parasites of the lungs and tracheae, expressed normally through coughing. (sputum examinations)

   b. *nasopharyngeal secretions*—utilized by parasites of the nasopharynx, expressed normally through sneezing. (nasopharyngeal washings)

   c. *saliva*—utilized by the parasites of the mouth cavity, transmitted by kissing, transfer of prechewed foods, or common use of cups and feeding utensils. (buccal or gingival scrapings)

   d. *seminal fluid and/or vaginal secretion*—utilized by the parasites of the genital tracts and transmitted by coitus. (vaginal-urethral scrapings and lavages)

3. *the blood (and lymph)*: utilized by parasites of the blood cells (both erythrocytes and leucocytes), the reticulo-endothelial system, the lymphatic system, and associated glandular and sinusoidal fixed cellular elements, and a variety of other tissue para-

sites (e.g. heart muscle, cutaneous lesions, epithelial lesions) all of which have an infective stage in the circulating blood or lymph. Mode of transmission usually by blood-sucking insects and other arthropods, but also found occasionally in the feces, urine, nasopharyngeal secretions and cutaneous exudates, from which sites (in the hemoflagellate protozoa) they may also be infective. (blood smears)

4. *the urine:* utilized by parasites of the kidney, bladder, blood sinusoids of these organs, and of some blood parasites themselves. Transmission normally through direct contamination of soil or water. (urine examinations)

5. *the skin:* utilized by parasites living intra- or subcutaneously, e.g. *Dracunculus medinensis,* the Guinea worm or Firey Serpent of the Near East; *Onchocerca volvulus,* the blinding filarial worm; and a variety of myiasis producing flies—transmission either by blood-sucking insects or by direct expression from the skin. (skin scrapings)

For diagnosis of ectoparasites, the parasite or its eggs are found attached to the skin or hair, are examined for in skin scrapings, looked for on the clothing, or, after recognizing the typical symptoms of the bite, are looked for in the habitats of the pest. Transmission is usually by direct contact in the permanent parasites; the temporary parasites go from host to host under their own volition.

For diagnosis of somatic tissue parasites (e.g. trichinae, cysticerci of Taenia tapeworms, hydatid cysts of Echinococcus, sparganum larvae of tapeworms) and also for diagnoses of parasites of the reticulo-endothelial system, blood, and lymphatic tissues which normally use the blood via blood-sucking insects as their portals-of-exit but which may be difficult to find in the blood at any one time, direct tissue biopsy is resorted to.

1. *Biopsy:* the removal of pieces of tissue containing the parasite from the living host.
   a. excised pieces of muscle (muscle biopsy)
   b. scrapings from cutaneous lesions (skin biopsy)
   c. slices of skin (skin biopsy)

2. *Puncture:* the removal of tissue pulp material or cavity fluid from the living host.
   a. spinal puncture
   b. splenic puncture
   c. liver puncture
   d. ascitic fluid aspirates
   e. gland puncture
   f. sternal puncture

In addition to: a) the direct examination of the portals-of-exit

and b) tissue biopsy and puncture there are additional laboratory confirmation techniques performed on the subjects and the parasites either recovered from the subject or their presence suspected or confirmed by visual signs.

1. *Cultural Methods:* the culturing of scanty parasitic material for its reproduction into sufficiently large populations for finding and confirmatory diagnosis; for the production of antigenic material; or for the study of their physiological and metabolic requirements and activities. Although some arthropods and some nematodes can be and are cultured, most of the techniques are developed for protozoan parasites.
   a. *in vitro cultures:* used very frequently but not routinely to diagnose intestinal protozoa and the hemoflagellates; cultures mainly used, however, for immunological or physiological studies. Most species of parasitic protozoa can be cultured.
   b. *in vivo cultures: in vertebrate hosts:* cultures, mainly protozoa but also of many nematodes and arthropods, are made in or on highly susceptible laboratory, domestic, and field animals, where they grow better than in humans. A sensitive diagnostic method but very slow. In effect, using another animal to screen out the contaminating organisms while the few original parasites in the inoculum gain a chance to grow luxuriently in a natural or highly susceptible foreign host, e.g. hamsters and *Leishmania donovani*. *in insect hosts: Xenodiagnosis.* The saliva injected with the bite of many blood sucking insects is highly attractive to the infective stages of the parasites they transmit and there is a swarming of these organisms (microfilariae, hemoflagellates) around the site of puncture. The insect thus not only concentrates the organisms but also after ingestion permits the parasite to reproduce freely or complete its life cycle. A sensitive diagnostic method. Of course, clean, uninfected insects which are natural hosts of the parasites must be used, e.g. extensively used for the diagnosis of *Trypanosoma cruzi* infections, utilizing the Triatomid bugs, *Rhodnius prolixus.*
2. *Serological testing*
   a. complement fixation
   b. skin reactions
   c. precipitin tests

   The methods are highly sensitive but have numerous drawbacks: they do not become positive until relatively late in the infections; they are not specific (there are extensive cross-reactions between the different groups, even phyla, of helminths); they remain positive for long periods after the cessation of clinical

symptoms and expulsion of the parasite; they are difficult to control.

Except for a few exceptions, most parasitic infections are far more easily diagnosed by finding the parasite itself.

Tests, when properly performed, controlled, and evaluated are of value in the following diseases: schistosomiasis, hydatid cyst and cysticercosis, trichinosis, filariasis, and amebiasis.

3. *Radiological Examination:* The use of X-ray or fluoroscopy for the detection of gross lesions, encysted or calcified parasites, or occasionally the living parasites themselves. These are of value in only a limited group of infections—echinococcus hydatid cysts, cysticercosis of Taenia tapeworms, trichinosis, and occasionally in obstructed or impacted intestine, appendix, or ducts as *Ascaris*.

4. *Clinical Symptoms:* A valuable adjunct in determining severity of disease or infection but of little value in diagnosis of the parasitic infection in itself, except in a few cases where the symptoms are pathognomonic of the infection. Usually, since the range of responses the body has to stimuli is rather limited, too many other diseases show similar symptoms, although not usually the same combination of symptoms.

On dead subjects at autopsy; on injected laboratory animals used for culturing the suspected recovered organisms; or in epidemiological and life cycle studies of the parasite in laboratory or wild field animals, additional techniques are available for confirmatory diagnosis and parasite recovery which are not available on the living patient.

1. *Examination of Internal Organs*—for parasites and pathologies.
   a. *Gross examination:* The animal is completely autopsied. Both the exterior surface of the skin and hair and the interior surface of the subcorium are examined as the skin flaps are folded back; the peritoneal walls and the loops of the omentum and its circulatory network are examined by transmitted light; the alimentary canal from oesophagus to rectum is removed, split longitudinally and minutely examined, its contents are floated in water, and the tract is finally washed clean with water; the liver, gall bladder, spleen, kidneys, and their ducts and associated glands are all examined grossly, sliced at different levels, and the ducts split lengthwise; the lungs and heart are removed and split or teased apart; the brain, spinal cord and meninges are removed and sectioned at different levels; pieces of muscles from the diaphragm, intercostal

region, tongue and extremities are all removed. Finally the atrial cavities, mouth, nasopharynx, bladder, genital systems and their ducts and openings are split and examined. The larger parasites are removed and preserved for identification; full note is kept of all lesions observed, and representative pieces of tissue are preserved.

b. *Histological Study:* Representative lesions and their surrounding unchanged tissue are preserved in any of the approved fixative agents, and prepared for sectioning in the regular manner. Suitably stained microscopic slides are prepared of the lesions and their contained parasites.

2. *Tissue Smears:*
   a. *brain and meningeal smears:* mashed impressions and/or smears made from pieces of the brain surface and meninges.
   b. *liver impressions:* made from the freshly cut surfaces of transections of the liver. *Viscerotomy*—use of the viscerotome, a specially designed knife to remove plugs of tissue from the liver, for shipment back to a central laboratory for examination. (Use is required in all Latin-American countries, on all people who die within three days after onset of any natural illness—mainly for the detection of yellow fever.)
   c. *splenic impressions:* made from the freshly cut surfaces of transections of the spleen.
   d. *heart blood smears*
3. *Body Cavity Exudates*
   a. *Peritoneal and pericardial cavity aspirations:* To find the parasites (numerous protozoans, e.g. *Toxoplasma, Leishmania*) or eggs or larvae of various helminths.
4. *Meat Inspections*
   a. Run routinely on all slaughtered beef, hog, and sheep meats intended for interstate shipment, for examination of tuberculosis, cysticercus bovis and cellulosae (and also non-parasitic pathologies). Muscles from various areas of the animal are examined.

In epidemiological surveys and life history studies on the parasite, additional studies are made on the free-living stages, on the encysted stages, and on the stages in the intermediate hosts and vectors. Among these the most important are:

1. *Soil Examinations:* For the detection of eggs and larvae of nematodes, particularly hookworms, but also for the recovery of cysts.
   a. *aerial examinations:* to study migration of air-borne particles, exposed gelatin plates placed on undercarriage of airplanes flown at different altitudes.
   b. *dust examinations:* resistant egg and cyst stages (e.g. of

the human pinworm, *Enterobius),* commonly found in accumulated dust on furniture, around rooms, etc.

c. *water examinations:* for the discovery of cysts, cercariae, infective larvae, etc., of parasites which may be waterborne.

2. *Examination of Fruits and Vegetables:* For study of contained protozoal cysts, eggs and even infective larvae of nematodes. Usually made by careful washings of the surface of the foodstuffs, sedimentation of the collected solids, and microscopic examination of the sediment.

3. *Examination of Intermediate Hosts and Vectors:*

a. aquatic animals and plants for hominoxious fluke, tapeworm, and nematode infections. (Crabs and crayfish, snails, leeches; fish; microcrustaceans (*Cyclops,* etc.); and aquatic vegetables are dissected to find the metacercarial stages; the sporocyst-redia stages; the procercoid and plerocercoid stages; etc.)

b. insects and other arthropods: e.g. dissection of mosquito's salivary glands and stomach wall to determine vector efficiency of % infectivity in malaria; cultural inoculations of triturated insect tissues.

4. *Carrier Examinations:* Routine checking of food-handlers as carriers of *Endamoeba histolytica,* by fecal examinations, and examination of fingernail scrapings.

Keep in mind the following facts:

1. Parasitic infections can only be verified by finding and positively identifying the parasite itself or its reproductive stages—hence the stress placed on correct diagnosis. Lack of diagnosis or incorrect diagnosis can lead to grave harm or useless or incorrect therapy.

2. Not every specimen of a parasite can be identified positively every time. If the specimen under view is not typical, move on and look for more typically diagnostic specimens.

3. Speed and accuracy in diagnosis comes only with long years of experience.

4. Take into consideration other accessory information in making the diagnosis, e.g., the source of the specimen, indicating the location of the parasite within the body.

5. There are numerous variations of all the basic techniques. Almost every laboratory technician or individual clinician develops his own variants and short cuts. Highly recommended methods in the hands of one investigator may give very poor results in the hands of another. Develop one approved method and stick to it. In the great majority of cases it will be adequate. Use supplementary techniques when indicated.

6. Practice, systematization, and accuracy are the most important points in satisfactory laboratory diagnosis.

2. *Deep Freezing as a Method of Preservation of Parasites.*

Frequently it is desirable to preserve interesting parasitological and pathological materials in their natural condition for teaching purposes or later study. This is especially necessary for rare material which is not always available in a fresh state.

In past years the problem was met by museum preservation. That is, fixing the material carefully with special fixatives like Keyserling's fluid, and mounting in optically clear glass museum jars. These jars are almost unobtainable now and are prohibitively expensive. Moreover, there was always danger of the liquid's drying out so that it had to be renewed constantly.

Now, with the widespread use of deep-freeze lockers, refrigerators, and cabinets, a simpler and more satisfactory tool is available. The specimen is merely wrapped in waxed paper or placed in a closed carton to prevent dehydration and placed in the deep-freeze chest or compartment of a refrigerator.

The specimens are frozen rapidly. In the frozen state, they do not look natural, but have the appearance of crystalline ice. However, they are preserved. When thawed naturally by merely removing from the chest they resume their original natural appearance. This procedure may be repeated indefinitely and the materials may be stored for an indefinite length of time.

As a teaching aid in parasitology, we have used this with success on fecal parasites. As different types of stools come into the laboratory—liquid diarrhetic, mushy, formed, black, blood-flecked, mucoid-streaked, children's, etc. representing the range of stool types, they are deep-frozen and preserved for class. They are later thawed out and at one time, the whole range can be demonstrated. In the same way, when stools containing parasites—*Ascaris, Trichiurus, or Taenia or Diphyllobothrium* tapeworms, or flukes such as *Clonorchis* or *Schistosoma*, or hookworm eggs, etc., come into the laboratory they are preserved.

Wet smears made from these are the equivalent of fresh preparations; the students prefer them to the older formolized material.

The thinner walled hookworm eggs are, generally, satisfactory, although their numbers are reduced in the $ZnSO_4$ floatation methods.

Cysts of protozoa, unfortunately, are not too well preserved. They all but disappear from the feces, even after $ZnSO_4$ floatation. Trophozoites are killed and distorted outright.

Gross tissue preparations showing lesions are beautifully preserved—they resume their original color upon thawing.

Where teaching has to be done, deep-freezing is recommended in place of formolized preservation.

3. *The P.V.A.-Fixative Technique for Preservation of Intestinal Protozoa.*[2]

One of the rules of a well-run diagnostic laboratory is that which requires all stools being submitted for fecal examination of parasites to be brought into the laboratory within 30 minutes of being passed, especially if the stool is mushy, diarrhetic, or liquid. Formed stools can be left unstudied longer, a day or more, or even several days. Moreover, formed stools can be preserved in the refrigerator for some days or fixed and preserved with 10% formalin and studied when time is available. The formed stools contain the rounded up protozoan cysts which are fairly resistant and can stand such treatments and still yield satisfactory diagnostic criteria.

Liquid stools, however, contain the trophozoites of the intestinal protozoa, especially the amoebae and flagellates. These are the mobile, feeding stage, and are very delicate. They round up and die within 1—2 hours and can no longer be specifically identified. Since it is the diarrhetic stool which worries the clinician most and makes him think of amebiasis or amebic dysentery, it was this kind of stool which gave the most trouble. When the physician was not located near a laboratory or especially when he was located in the smaller towns without laboratory facilities, proper laboratory diagnosis presented grave problems.

The P.V.A.-Fixative technique, introduced by Goldman in 1948 and 1949, now remedies that difficulty and affords many other advantages.

It combines one of the best cytological fixatives for protozoa (Schaudinn's fluid—2 parts saturated aqueous solution of mercuric chloride, plus 1 part 95% ethyl alcohol, 5% glacial acetic acid, plus 1½% glycerol) with the synthetic water-soluble, colorless, transparent resin, polyvinyl alcohol. This resin, or plastic, forms a tenacious film over the specimen which prevents the specimen and the parasites from drying out, yet is permeable to all the dehydrating agents and stains, and does not interfere with microscopical inspection. The films are resistant to alcohol, xylol, ether, acetone, and oils. Normally, there is 5% by weight of the dry P.V.A. dissolved at 75° C in the Schaudinn's fluid.

The polyvinyl alcohol comes in several grades, each with a different viscosity. The recommended grade is No. 90-25, sold under the trade name "Elvanol" by E. I. DuPont and Company.

The Schaudinn's fluid, then, fixes the trophozoites of the amoebae and the flagellates like *Giardia, Trichomonas,* etc., in their natural

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[2] Ref.: Brooke, M. M., Goldman, M., and Johnson, S. A.: (1949) "Polyvinyl Alcohol-Fixative as a Preservative and Adhesive for Protozoa in Dysenteric Stools and Other Liquid Materials." J. Lab. & Clin. Med., 34:1554-1560; Nov., 1949. (Contains references to other papers.)

condition, and the polyvinyl alcohol maintains them in that state until they are smeared on slides and stained. In addition, the P.V.A. acts as an adhesive and fixes the parasites to the slide.

The procedure is as follows:

In practice, two 15 cc. vials are supplied to the physician. One contains the P.V.A.-fixative mixture, the other 4% formaldehyde (10% formalin). About 4 cc. of liquid diarrhetic or mushy stool is placed in the P.V.A. vial and *thoroughly* mixed. This thorough mixing is important—use an applicator stick. If the stool is formed, use a piece the size of a pea, macerate with the solution, and mix thoroughly. Another sample of the stool is placed in the formalin vial and also mixed well. The vials are then sent off to the laboratory. An intelligent picking of representative sample sections of the stool for preservation is important. Pick blood-flecked areas, mucous strands, etc. Areas of scraped mucosa obtained by proctoscopic examination can also be used.

Smears for staining can be prepared immediately or months later. Spread a drop or two of the P.V.A.-fixative-stool mixture on a microscope slide and *allow to dry thoroughly!!* Care must be taken not to have the smear too thick. This drying of the smear permits the resin to form a film which is resistant to further manipulations. Drying may be hastened by heating on a hot plate, in an incubator, by being blown with warm air, etc.

Any of the variety of iron-hematoxylin staining procedures can be used. Of these there are a great number described—and most of them are suitable. Any stain which can be applied on an ordinary fecal smear can be applied to a P.V.A. resin covered smear. The only difference is that it is necessary to increase slightly the time in each step from the normal 2 minutes to 4 minutes and increase by 50% the length of time in the stain. The resin films are fully permeable to all the stains, but retard their passage slightly, hence the increased time.

There is one great advantage to this technique. AT ANY TIME THE SLIDES MAY BE REMOVED FROM ANY OF THE STAGES IN THE STAINING PROCEDURE AND PERMITTED TO DRY WITHOUT DAMAGE. Compare this with the usual warnings that the slides must never be allowed to dry at any stage with ordinary smears.

The ordinary procedures of staining are used. After the slide has dried it is placed in 70% alcohol to which Lugol's iodine solution has been added to remove the mercuric chloride crystals, then into 50%, then into 30% alcohols and then into running tap water, each stage for from 2 to 4 minutes. Then, depending on the technique of hematoxylin staining being used, it goes into the mordant, e.g., 2% iron-alum solution (aqueous) at 40° C. for 2 minutes; wash in running water, then stain in 0.5% hematoxylin solution (aqueous) for 2 minutes, wash in running water,

and destain (differentiate) in cold iron-alum solution, wash in water, and successively immerse smears in 50%, 70%, 90%, and absolute alcohol, and xylol for 2 minutes each. Mount in balsam or other mounting medium.

Although it is preferable to mount the smears under a cover glass, it is not necessary and uncovered films can be examined with oil immersion objectives.

The method is not recommended for protozoan cysts or helminth ova or eggs and, although it can be used, the use of the wet-mounts made from the formolized stool is preferable. Here is where the second vial, containing the 10% formalin comes into play. Make a wet smear as usual with the suspension from this vial. The cysts and eggs are highly refractile and can be easily spotted—this refractivity is lost in the resin film smears. However, they can be identified in this preparation, although not as easily picked out.

One other technique is available when using the P.V.A.-fixative method. That is, it can be used with advantage even when the fresh stools are brought into the laboratory. The mixing of the stools and the P.V.A.-fixative can be made directly on the slide. A drop of the dysenteric stool or other material is placed on a microscope slide and mixed with 3 drops of the P.V.A.-fixative. The mixture is then smeared over two-thirds of the slide surface and allowed to dry thoroughly (preferably overnight at 37° C.) Dried smears remain satisfactory for staining for many months. The advantage is the increased tenacity with which the smears are held to the slide—specimens are not lost—and also the fact that the slide can be dried at any time.

The question might be asked, is this technique suitable for concentration methods? The answer is "No." The usual concentration methods can't be used. Moreover, concentration methods are *not* applied to liquid stools since these contain few cysts. They contain mainly trophozoites. The formed stools which contain the cysts are either not preserved or are formalin preserved. On these, concentration methods can be used.

4. *Use of Detergents in Staining Blood Films to Prevent Transfer of Parasites.*[3]

For the past 30 years in malaria survey work and in the clinics where large numbers of blood smears are examined for malaria parasites, it has been the custom to resort to mass staining of the slides. (The thick-film technique is used mainly.) That is, 25 or 30 slides will be bound together with rubber bands at one end, each slide separated from the next by a small cardboard divider. The slides are then all immersed together in the stain and stained at one time. (The level of the stain is kept suffi-

ciently low so that the dividers themselves are not wet.) In this way, a great amount of routine handling of the slides is avoided and the slides are all stained uniformly. There is a great saving in the stain also as the same batch can be used for a whole day to treat innumerable slides.

The mass staining method had much to recommend it and was widely adopted throughout most tropical countries and the Southern U. S.

In 1948, however, Brooke and Donaldson made a most disconcerting discovery. They found that when known negative smears were stained in the same batch with malaria-positive bloods, isolated parasites and infected cells were found on the slides with the known uninfected bloods!! This led to erroneous diagnoses—false positives. Not only could people free of malaria be recorded as being malaria-infected but in areas where several species of malaria parasites are found, there could be confusion in specific diagnosis of the species involved. Records of double or even triple malaria infections could be recorded where normally the individual was infected with only one species. In making their study, these authors worked with known negative bloods and known positive bloods handled in the routine way. They performed additional tests, however, and slides of blood smears from birds were stained along with the human blood slides. Since the red cells of birds are oval, large, and nucleated they are easily distinguished. The human blood slides were found to have picked up the nucleated red cells from the stain!! Examination showed that, in most cases, flakes of blood flake off the slide and fall into the surface of the stain where they float on the surface and attach themselves to neighboring slides. This was especially true when slides faced each other with smear sides opposing.

If nothing had been done, this could have meant the end of mass staining procedures and perhaps the ending of mass malaria surveys because otherwise the labor involved in handling the slides individually would have been too tedious.

However, the same authors have worked out, in 1950, the remedy. By adding 0.5% of the surface active agent Triton X-30 (manufactured by Rohm & Haas Co., Philadelphia) to the diluted Giemsa stain (1:50) which they used in their staining they reduce the surface tension of the solution from 58.8 dynes per cm.[2] to 30.2 dynes per cm.[2] The particles which flake off the

[3] Refs.: Donaldson, A. W., and Brooke, M. M.: (1950) "Effects of Various Modifications of a Mass Staining Procedure on the Transfer of Malarial Parasites Between Blood Films." J. Nat'l Malaria Soc., 9:239-247, Sept., 1950.

Brooke, M. M., and Donaldson, A. W.: (1948) "Transfer of Malarial Parasites Between Blood Films During Mass Staining Procedures." Public Health Reports, 63:991-1004.

Wilcox, A.: (1943) "Manual for the Microscopical Diagnosis of Malaria in Man." Nat'l Institute of Health Bulletin No. 180.

slides now fall to the *bottom* of the stain, and there is little or no transfer of infected cells from an infected slide to a non-infected slide. This prevents false positives from appearing.

Moreover, the slides are cleaner, there is less precipitate deposited, and the staining seems sharper. Hence the detergent has no effect on the staining quality of the stain. Undoubtedly other wetting agents can be used in addition to Triton X-30.

5. *The Velat-Weinstein-Otto Stain for the Rapid Differentiation of Trophozoites of Intestinal Protozoa in Fresh Wet Preparations.*[4]

The specific identification of trophozoites of the intestinal amoebae is an important phase of stool examinations. Upon it depends the correct diagnosis of amebic dysenteries, and the preventing of readings of false positives with their attendant consequences.

However, such identification is not easy. Usually size, type of pseudopodia, movement, cytoplasmic inclusions, etc., are used to identify the forms of fresh, wet preparations. In fixed and stained preparations, the character of the nucleus is the most important and the actual structure on which confirmed diagnosis is made. Usually hematoxylin-stained fixed smears are made for this confirmatory diagnosis. But the procedures are time consuming and previously when wet smears were made before the introduction of the P.V.A.-fixative technique, many of the specimens were lost from the slides as they were being stained.

The goal, therefore, has been the development of a simple, "wet-preparation" stain; that is, one which would stain the nuclei (and cytoplasm) of the organisms without killing them in the preliminary wet smears with sufficient accuracy so that the nuclear morphology could be distinguished.

Until last year, Quensel's stain developed in 1935 and consisting of a combination of Sudan III and methylene blue was widely used. With this stain, the feces are mixed with the stain and let stand 20 minutes before examination. The cytoplasm stains a light blue and the nuclei a dark blue. Ciliates, flagellates and cysts do not stain. Iodine, which is used in many modifications on cysts, can not be used on trophozoites because it destroys them. Many other vital dyes had been tried, such as neutral red, Janus green, etc., but without success.

A highly satisfactory stain and procedure has now been developed.

This stain, developed by Velat, Weinstein, and Otto is a buf-

[4] Refs.: Velat, C. A., Weinstein, P. P., and Otto, G. F.: (1950) "A Stain for the Rapid Differentiation of the Trophozoites of the Intestinal Amoebae in Fresh, Wet Preparations." Am. J. Trop. Med., 30:43-51.

fered, aqueous solution of the precipitate obtained upon the interaction of hematoxylin and crystal violet. The procedure of manufacture of this stain is highly complex and the original paper must be referred to for the details. In brief, however, a 2.5% solution of crystal violet and a 1% solution of hematoxylin are reacted in the presence of triethanolamine and a precipitate forms. This is washed several times and dried. The precipitated stain is then dissolved in varying mixtures of acetic acid-sodium acetate solutions which act as both the solvent and buffer the solutions at various pH's from 4.6 - 5.4. Critical staining is achieved at these pH's.

So far, the stain is not manufactured commercially but will undoubtedly soon be made available.

Use of the stain: trophozoites are stained by mixing a small quantity of fecal material into 1 or 2 drops of the stain on a clean slide. A number one cover glass is applied and let stand a few minutes (3-5 minutes). The preparations are examined as a wet mount in the usual manner. The organisms are located using low power (100X), studied under high dry (430X) and confirmed under oil immersion (970X).

With this stain, the particulate matter on the slide with the exception of the protozoa are stained a light purple color, against the faintly pink background of the solution. The trophozoites are refractile and easily picked out. Their nuclei are seen to be prominently stained. The chromatin granules and the karyosome are stained a bright purple-black which contrasts markedly with the cytoplasm which is stained a light purple color. Cytoplasmic inclusions are stained with varying intensities.

In ordinary saline mounts, the nuclei of *E. histolytica* are not visible, and those of *E. coli* only faintly so. With this stain, the various species can be easily differentiated by the characteristics of their nuclei.

This stain is only suitable for trophozoites. Mature amebic cysts do not stain (they are adequately stained with the various iodines), but the dye occasionally penetrates into the immature or young cysts.

In the flagellates, the flagellae and undulating membranes become more readily visible, as do the fibrils, chromatin, and nuclear membranes. They all stain varying degrees of purple-black.

Other fecal structures like yeasts, Blastocystis, macrophages, plant cells, bacteria, etc., also stain to varying degrees but are readily distinguishable.

The stain can also be used on blood parasites—microfilariae of filaria worms and trypanosomes. The nuclei and G cells of the former stain sharply; in the latter, the kinetoplast, volutin granules, nuclei, and undulating membranes all stain well.

6. *Cultural Methods as a Routine Diagnostic Procedure in Isolating Trichomonas vaginalis.*[8]

Trichomonas vaginalis is a common protozoan parasite of the vagina of women and, less commonly, of the urethra of males. Depending on the group surveyed and the methods employed, from 15 to 45% of women are infected. Perhaps the average of 30% is nearly correct.

This parasite is associated with a definite pathologic lesion—a vaginitis—which is highly irritating. There is a great deal of controversy over whether *Trichomonas* is the actual causative agent, is a secondary invader, or works in conjunction with concomitant bacterial infection to produce the clinical syndrome.

Parasitologists, for the most part, doubt its actual pathogenicity. Clinicians, on the other hand, are definitely convinced that they are the causative agents, their great number, active whipping movements of their flagellae, etc., causing a continuous irritation which causes a hyper-secretion from the vaginal mucosa. The vaginitis is highly irritating, causing varying degrees of actual pain and discomfort, and an odoriferous secretion which is embarassing. The infection is highly resistant to treatment and may become chronic. Reinfection is easy. There are no satisfactory therapeutants so far developed, merely partial therapeutants and palliatives.

In the obstetrical and G-U clinics, routine microscopic examinations of the vaginal secretions are resorted to, in order to identify the etiologic agents of the vaginitises.

Usually, where the organisms are numerous, and they occur by countless millions, a simple smear made from exudate which remains on the vaginal speculum or direct swabbing from various areas of the vaginal wall, especially near the uterine ostia, onto a wet mount in saline on a microscope slide is sufficient to establish the diagnosis. The trichomonads are easily seen under low power, actively swimming around.

In over 80% of the cases, this procedure is sufficient. However, where the secretion is scanty or where the organisms are few, direct microscopic examination may not be sufficient. In this case, routine culture of the exudate should be resorted to.

A swab is introduced into the vagina, touched to various suspected areas, and dropped into a tube of culture medium. Growth in 48 hours is sufficiently high to show positive or negative results on microscopic examination of the culture sediment.

This cultural method of diagnosis gives positives in about 20% of the cases which would normally be called negative on direct microscopic examination. Hence it is more sensitive and more reliable.

Numerous culture media have been developed for this type of diagnosis. Many of them are to be found in Craig's handbook, "Laboratory Diagnosis of Protozoan Diseases."

For the most part the media consist of Locke's solution (a balanced physiological Ringer's saline), and blood serum (from a variety of animals) with or without agar. They can be made bacteriologically sterile by adding 250-1000 units of penicillin per cc. at the time of inoculation. Dilute methylene blue may be added to indicate the aerobic zone. The agar increases viscosity and aids the cysteine in maintaining an oxygen-free condition in the major portion of the culture medium.

These media, while quite suitable, give populations of 1500-2500 organisms per cubic millimeter. Dr. Hartman of the Loyola Medical School has perfected a medium (unpublished) which yields 12-15,000 per cu. mm.

While any of the media can be made up in the individual laboratories, Microbiological Associates, Inc., of Flemington, N. J., have prepared an excellent medium which comes already tubed and ready for use. This medium, called STS (for Simplified Trypticase Serum) is a liquid which contains maltose, agar, trypticase, cysteine, methylene blue, water, and sterile human serum, all adjusted to a pH of 6.0. It comes in tubes containing 8 cc. each. This medium is a modification of Johnson's CPLM medium of 1943 which was one of the first based on a rational analysis of cultural and metabolic requirements.

I would like to conclude this section by recommending that cultural isolations be used on all cases of vaginitis in which organisms can not be identified by direct microscopic examination, and on cases which have been under treatment, to determine final clearance of the organism.

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[8] Refs.: Craig, C. F.: (1948) "Laboratory Diagnosis of Protozoan Diseases." Lea & Febiger, 2nd Edition, pp. 140-145.

Johnson, J. G.: (1947) "The Physiology of Bacteria-Free *Trichomonas vaginalis*." J Parasit., 33:189-197.

Lash, J. J., and Belt, E.: (1949) "Cultural Methods for Diagnosis of Trichomonad Infections." Am. J. Obstet. & Gynec., 57:981-983.

Wenrich, D. H.: (1946) "Culture Experiments on Intestinal Flagellates. I. Trichomonad and other Flagellates Obtained from Man and Certain Rodents." J. Parasit., 32:40-53, Feb. 1946.

Trussell, R. E., and Johnson, G.: (1943) "Experimental Basis for the Chemotherapy of *Trichomonas vaginalis* Infestations." Proc. Soc. Exper. Biol. Med., 54:245-249.

Sprince, H., and Kupferberg, A. B.: (1947) "The Nutrition of Protozoa. I. A Simplified Medium for the Investigation of Unknown Factors in Blood Serum Essential for the Sustained Growth of *Trichomonas vaginalis*." J. Bacteriology, 53:435-439, April, 1947. II. The Separation of Human Blood Serum into Two Fractions, both Essential for the Sustained Growth of *Trichomonas vaginalis*." J. Bacteriology, 53:441-447, April, 1947.

Microbiological Associates, Inc., Coral Gables, Florida: "STS Medium (Simplified Trypticase Serum) for the Cultivation of Trichomonads."

Kupferberg, A. B., Johnson, G., and Sprince, H.: (1948) "Nutritional Requirements of *Trichomonas vaginalis*." Proc. Soc. Exper. Biol. Med., 67:304.

Johnson, G., Trussell, M., and Jahn, F.: (1945) "Isolation of *Trichomonas vaginalis* with Penicillin." Science, 102:126.

7. *Cultural Methods for the Routine Diagnosis of Endamoeba Histolytica Infections.*[6]

As a general rule, it may be stated that in all cases of amebiasis caused by *Endamoeba histolytica,* a sufficiently thorough examination of fecal smears, repeated up to six times if necessary, and utilizing salt purges to obtain diarrhetic stools, will reveal the presence of the causative organism.

However, that is not always so easily accomplished. Concentration methods by $ZnSO_4$ floatation may fail to show cysts; patients may not return to be examined a sufficient number of times to assure a statistically valid statement that they are negative (2 examinations will show 40% more positives than 1 examination; 3 examinations will only detect 85% of all positives; as many as 6 stool examinations may be necessary).

The disease, amebiasis, is so important and is the cause of such a high percentage of ill-defined clinical symptoms that it is necessary to rule out this organism as a cause of the disease. *In all cases where E. histolytica is present, there is a definite pathology of amebiasis.*

Hence, where amebiasis is suspected and the organisms can't be identified in fecal smears, culturing the organisms is indicated.

Many main types of media are available for this; the most widely used are

1) Boeck & Drbohlav's diphasic medium
2) Shaffer & Frye's Transparent medium
3) Balamuth's medium
4) Nelson's Alcoholic Extract Medium (one of the best for primary isolation)

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[6] Shaffer-Frye Transparent Medium [Shaffer, J. G., Ryden, F. W., and Frye, W. W.]: (1948): "Studies on the Growth Requirements of *Endamoeba histolytica.* III." Am. J. Hygiene, 47:345-350. (1949): "Studies on the Growth Requirements of *Endamoeba histolytica.* IV." Am. J. Hygiene, 49:127-133.

Balamuth's medium: Balamuth, W.: (1946) "Improved Egg Yolk Infusion for Cultivation of *Endamoeba histolytica* and other Intestinal Protozoa." Am. J. Clin. Path., 16:380-384.

Craig, C. F., and Faust, E. C.: (1951) "Clinical Parasitology." 5th Edition, Lea & Febiger, Philadelphia, pp. 867-871.

Anon: (1948) "The Preparation of Modified Boeck & Drbohlav's Medium." CDC Bulletin (USPHS, Atlanta, Ga.), Oct.-Dec., 1948.

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Hallman, F. A., Michaelson, J. B., and deLaMater, J. N.: (1950) "The Cultivation of *Endamoeba histolytica* in a Defined Medium." Am. J. Trop. Med., 30:363-369.

deLaMater, J. N., and Hallman, F. A.: (1947) "Studies on the Culture of *Endamoeba histolytica.*" Proc. Soc. Exper. Biol. Med., 65:26-29.

Laird, R. L., Drinnon, V. P., and Davis, A. B.: (1949) "Routine Culture Methods in Diagnosing *Endamoeba histolytica.*" J. Nat'l Malaria Soc., 8:198-201 (a very important paper—shows advantages of culture over microscopic fecal smears).

Spingarn, C. L., and Edelman, M. H.: (1948) "The Use of Streptomycin in the Cultivation of *Endamoeba histolytica* from Stools." Am. J. Trop. Med., 28:825-829.

Phillips, B. P.: (1950) "Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi.*" Science, 111:8-9.

5) St. John's medium
6) Cleveland-Collier's medium

There are numerous modifications of these. For the most part, the media can be classified into a) diphasic types—that is, those in which there is a solid substrate overlaid with a liquid; and b) the all-liquid media.

The major ingredients of these media are:

1) Use of egg products (either yolks alone or yolks with their whites) combined with balanced salt solutions (Locke's or Ringer's) and with the addition of serum or liver extracts for enrichments.
2) Use of agar slants containing buffered liver extracts.
3) Use of liver extracts.

In almost all cases, the addition of particulate rice starch is necessary to stimulate or maintain growth.

Balamuth's medium is an all-liquid aqueous extract of egg yolk and liver in balanced buffered salt solution. This is made commercially by DIFCO and put up in sterile tubes, ready for immediate inoculation. For laboratories which do not have a lot of diagnostic work, this is both a practical and an economical item to use.

The growth of bacteria in the culture is almost necessary for adequate growth of the Endamoeba—in routine culturing. Experimentally, there are strains which will grow with dead bacteria; with bacterial filtrates; or with protozoans like *Trypanosoma cruzi* in place of bacteria—to supply the necessary growth factors. Progress is being made on completely synthetic media which will contain all the accessory growth factors, but no such media have as yet been developed which are satisfactory.

Streptomycin or penicillin are frequently added to the culture media at the time of inoculation to keep down an overabundant bacterial growth.

The actual technique of cultivation is very important, as is also the experience an individual has with a particular medium. The following points should be kept in mind.

1. Fecal material to be cultured should be freshly passed—the media will start cultures either from trophozoites or cysts.
2. Ordinary aseptic technique must be used to prevent gross bacterial contamination and overgrowth.
3. Large size inocula must be used—0.5 cc. of liquid stools or pieces the size of a pea from solid stools. Both these must be intimately mixed with the liquid overlay of the media.
4. Culturing protozoa differs from bacterial culture in several important respects: large quantities of inocula must be used; when transferring, large amounts of transfer must be used—use of a platinum loop is useless; use a large-bore pipette in all transfers.

5. In examining the sediment for growth of protozoa—a pipette should be used for withdrawing one or more large drops of sediment from either the bottom of the liquid or from the base of the liquid-slant junction—they do not grow in the liquid portion and it is useless to examine here.

6. Populations never build up to those in bacterial cultures—hence more liquid has to be examined more carefully.

7. Culture for 24 hours and examine—if negative, culture for an additional 24 or 48 hours. All cultures are maintained at 37° C.

8. In maintaining *E. histolytica* subcultures, it is necessary to subculture every 48-72 hours.

The preparation of most of the types of media used is time consuming and complicated, but they can be made up in large batches and keep indefinitely once made up.

The details of the procedures can be found in the references given.

One of the best media for primary isolation is Nelson's alcoholic extract. Laird, Drinnon, and Davis have published some very interesting figures: they showed that in one group of 145 patients in which 18% (26 individuals) were positive for *E. histolytica*, only 20 (13.8%) of these were found positive on direct iron-hematoxylin staining of fecal smears and 25 (17.5%) were found positive by culture (using Nelson's medium). In another group of 350 patients, staining showed 28 positives while culturing with Nelson's medium showed 48 positives.

The conclusion is obvious—a good culture medium should be added as a routine procedure in the laboratory diagnosis of *E. histolytica*.

Advantages of culturing are numerous:

1) It is capable of revealing organisms when only a few cysts are present in the stools.

2) Cultivation reveals a greater percentage of positives than direct examination of one or two fecal smears.

3) Most important of all however, is the ability to culture stools several hours old. In these stools, the trophozoites have disintegrated sufficiently so as to be unrecognizable. However, their progeny will develop in the culture media, and these progeny will be typical of the species.

It is suggested, therefore, that culture materials be kept on hand and attempts made at culturing when positive diagnosis can not be made on morphological grounds, and the stools are negative on microscopic examination.

8. *Rapid Hematoxylin Staining Methods for Fecal Smears*[7]

For confirmatory diagnosis of intestinal protozoan parasites, it is necessary to find the organism and positively identify it specifically. For this, it is almost essential to use hematoxylin

staining to bring out the specific morphological characters necessary for this positive identification.

Many laboratories do not routinely make permanent fixed stained smears of their fecal examinations. They use only temporary mounts—saline and iodine-stained. When skillfully done, this is satisfactory to report obvious parasites but is entirely unsatisfactory for stools in which there are scant parasites, and in which the parasites are not typical (there are strains which do not show typical morphology). On the whole, negatives reported by these methods are not reliable.

Permanently stained fixed slides can be studied at length without fear of drying; at the leisure of the technician; and maintain as a permanent record the findings if at any future time they are called into question. Moreover, the general character of the stool can be told from stained slides—the presence of Charcot-Leyden crystals, fat bodies, etc., which are of value.

Under the older staining methods which required 24 or more hours to stain and then careful destaining and differentiation, it was a chore to stain slides permanently. Now, however, with the shorter methods, it is no trouble at all. Slides can be turned out and ready for positive and confirmatory diagnosis in a matter of a few minutes to an hour, depending on the technique used.

It is very little extra trouble to make one or two extra smears for permanent staining at the same time that the temporary wet mounts are made. Just drop these smears into the fixative—Schaudinn's fluid or formalin.

The older procedures were based on fixing in Schaudinn's, then passing through iodinized alcohol and a series of alcohols to water. Mordanting was done in ferric ammonium sulfate, which was washed out, staining in 0.5% aqueous hematoxylin, washing, then a destaining in the mordant, followed by dehydration and mounting.

Now, through the use of heat, or by combining the mordant with the fixative, or through the use of more rapidly working mordants or hematoxylin stains, the use of progressive staining instead of regressive destaining, and the use of dioxan instead of graded alcohols to dehydrate and clear, it is possible to make permanently stained slides in a fraction of the time formerly required.

[1] Tompkins-Miller technique: Tompkins, V. N., and Miller, J. K.: (1947) "Staining Intestinal Protozoa with Iron Hematoxylin-Phosphotungstic Acid." Am. J. Clin. Path., 17:755-758.

Craig & Faust: (1951) "Clinical Parasitology." 5th Edition, pp. 864-865.

Craig, C. F.: (1948) "Laboratory Diagnosis of Protozoan Diseases." 2nd Edition, pp. 49-65.

Ratcliffe, H. L., and Parkins, P. V.: (1944) "On the Use of Mallory's Phosphotungstic Acid Hematoxylin for Staining Intestinal Protozoa." J. Lab. & Clin. Med., 29:534-535. (Young & Felsenfeld's Hektoen Research Institute method is a modification of this.)

Noble, G. A.: (1944) "A Five-Minute Method for Staining Fecal Smears." Science, 100:37-38.

By Noble's method, for example, formalin and glacial acetic acid are used as the fixative and ferric ammonium sulfate as the mordant. These are mixed together and applied to the smear which is heated to steaming over an alcohol lamp; the stain added and heated to steaming (allowed to act only 3 or 4 seconds) and washed off and quickly dehydrated in dioxan. The completed slide is ready in five minutes.

9. *Use of Thedan Blue in Staining Blood Smears*[8]

This past year, National Aniline started the commercial marketing of Dr. H. C. Simons' Thedane Blue stains for the rapid identification of blood parasites.

Actually the stains, sold in two strengths called T-3 and T-5, consist of a mixture of methylene blue and saponin. The latter material, saponin, is used to completely lyse the red cells so that the parasites are left in a clear field. The methylene blue stains the parasites simultaneously.

The great advantage of the technique is its rapidity. Full directions and details of use are enclosed with each bottle of stain. Briefly, however, the technique consists of mixing loopfulls of the thedane blue stain with drops of blood on a slide without spreading. Then add a coverslip. The results can be examined immediately.

Trypanosomes, blood spirochetes, piroplasmas, bartonellas, bird malaria parasites, and the cresent gametocytes of *Pl. falciparum* all show up as intensely blue staining organisms against a clear background. They are all distinguished under medium power.

The stain is a monochromatic stain; hence there is no color differentiation.

For small volumes of blood, the more dilute saponin solution T-3 is used; for large quantities of blood, the equivalent of several thick drops, the stronger saponin solution, T-5, is used. In either case the results are the same. It should be noted that as much as 0.1 cc. of blood can be put in one drop at one time and completely cleared with the solution. The only objects of the blood which are not lysed are the leucocytes, reticulocytes, platelets, and the blood parasites.

For Leptospira (causative organism of Weil's disease or infectious jaundice), and syphilitic spirochetes from serum exudates, the dark field can be used with this stain. The parasites show up as bright red rodlets under low power, and their morphology is seen under medium power. This redness is caused by selective diffraction ("Berek effect"), not by the organisms actually being stained.

[8] Refs.: National Aniline Division, Allied Chemical & Dye Corp., 40 Rector Street, N. Y. C. 6, N. Y. Brochures and Data Sheets on Thedane Blue, Solutions T-3 and T-5.

In addition, serum, cerebrospinal fluid, lymph from lymph nodes, chancre exudates, or urine sediments (in leptospirosis) can all be examined under dark field.

When used for the purpose for which they were intended, that is, for rapid diagnosis in fresh blood preparations—not for morphological researches—the stains adequately fulfill their function.

10. *Methods of Pinworm Diagnosis*[9]

One of the most common parasitic infestations in children—but also common in adults, is that called enterobiasis or oxyuriasis caused by the small nematode worm, *Enterobius vermicularis* (commonly named *Oxyuris vermicularis* in the clinical papers). These pinworms or seatworms are found in from 10 to 50% (and occasionally up to 100%) of children and are particularly prevalent among institutionalized groups. The worms live in the caecum and appendix and the colon and cause pinpoint lesions where they attach. The most common and important symptom, however, is the severe psychic disturbance caused, irritability, insomnia, restlessness, and a pruritus ani, which may be so severe that the incessant scratching of the irritated anal region results in hemorrhage, eczema, and pyogenic infection.

Although the adult worms live in the intestinal tract and this is the portal-of-exit for their eggs, only in a small percentage of cases (5%) are the highly characteristic flattened eggs found in the stools upon a fecal examination. This is because the females migrate down to the anus, pass out of the anal sphincter (usually at night) and deposit their eggs on the skin folds of the perianal region. The worm then retreats back into the colon. Hence it is important in diagnosing the infection to search the perianal folds for the eggs (and, occasionally the adult female worms). Fecal examination alone can not be relied upon. A variety of techniques have been developed to facilitate the recovery of the eggs.

The older techniques of mere scrapings with a slide or applicator were replaced by the use of wet glass pestles. This in turn was replaced by the use of the N.I.H. (National Institute of Health) cellophane swab, in which a piece of colorless cellophane is wrapped around the end of a glass applicator stick, rubbed over the perianal region, and mounted directly on a glass slide. This in turn, in the past few years, has been re-

[9] Beaver, P. C.: (1949) "Methods of Pinworm Diagnosis." Am. J. Trop. Med., 29:577-587.
Brooke, M. M., Donaldson, A. W., and Mitchell, R. B.: (1949) "A Method of Supplying Cellulose Tape to Physicians for Diagnosis of Enterobiasis." Public Health Reports, 64:897-901.
Markey, R. L.: (1950) "An Anal Swab Method for Detection of *Enterobius vermicularis*." Am. J. Clin. Path., 20:493.
MacKeith, R., and Watson, J. M.: (1948) "The Diagnosis and Treatment of Threadworm Infestation." Practitioner, 160:264-270.

placed by the use of ¾" wide Scotch-tape strips. Each method, in turn, gave a higher percentage of positive findings than its predecessor.

There are a variety of Scotch-tape techniques, the use of which will be found in the references cited. The tape itself may be formed into a bow with the adhesive side out, or the tape may be firmed with a glass slide or a tongue depressor. After daubing over the infested areas, the tape is smoothed out, adhesive side down, on a glass slide and examined microscopically. There is a handy technique available wherein the tape is supplied to the physician already attached to the slide. One end is removed, folded over the edge of a slide and a tongue depressor, daubed around the perianal region, then replaced on the slide in the manner in which it originally came. A drop of toluene can be added to the slide under the tape to displace the air bubbles, clear it of epithelial cells and debris, and dissolve the adhesive so that the attached eggs stand out more clearly. This has been found by Beaver to increase the percent of positives by 24% and decrease the time of searching by 40%. The Clay-Adams supply company now markets flat cardboard mailing containers which hold a glass slide, the tape, and a tongue depressor which can be supplied to the physician as a unit and mailed back to the laboratory for examination after use.

There are a number of artifacts ("cellophane cells") in the tape which simulate eggs and these must be taken into consideration in making the diagnosis. These, however, are soon learned and can be neglected.

Although these techniques are highly satisfactory, research is not stopping. Markey has recently proposed the use of a cotton swab coated with a mixture of 1 part of paraffin and 4 parts of petrolatum. After the anal daubing, the coating on the swab is dissolved in a solvent (xylol), the eggs centrifuged down, and the sediment examined microscopically for *Enterobius* eggs. The technique has the advantage that the patient may perform the swabbing on himself in his home daily in the early morning before his first bowel movement and bring the swabs to the physician's office or laboratory in the stoppered glass tubes in which they were originally supplied to him.

It must be remembered that one negative tape does not establish the absence of the parasites. At least 6 or 7 negative examinations taken on alternate days are necessary before the patient can be stated to be negative or cleared of an infection after therapeutic measures.

11. *"An Aid to the Diagnosis of Helminths Parasitic in Humans" by Roudabush, R. L., and Romaniak, T.*[10]

I would like to call your attention to this little handbook

(price 25c) which has proven of great value. Although written in 1943, it is apparently little known among most laboratory technicians. However, it is excellent, and I recommend it to you all. It is a handbook of accurate photographs showing the eggs of all helminth parasites of man in their normal size relationships as seen under high dry of the microscope. It also contains some valuable points on technique.

The foregoing discussions which only scratch the surface in themselves do not, of course, exhaust the list of advances in diagnostic techniques which have been made. Time and space preclude, however, a discussion of a number of other highly interesting advances:

a. the use of phase contrast microscopy in direct diagnosis of blood, tissue, fecal, and cultural parasites.
b. newer polychromatic stains for staining blood parasites.
c. use of fluorescent dyes and ultra-violet microscopy.
d. Loughlin-Stoll Acid-Ether-Xylol (AEX) Technique for concentrating nematode and schistosome eggs in the feces.
e. all-purpose concentration techniques for stool parasites.
f. the Sabin-Feldman Dye Test for Toxoplasmosis.
g. immunologic tests for helminthic and protozoal infections.
h. photometer calibration methods for making quantitative helminth egg counts and estimating adult worm populations.

[16]Roudabush, R. L., and Romaniak, T.: (1943) "An Aid to the Diagnosis of Helminths Parasitic in Humans." Ward's Natural Science Establishment, Inc., Rochester, N. Y.

STUDIES BASED ON ERRORS OBSERVED IN THE USE OF ANTICOAGULANTS IN BLOOD CHEMISTRY DETERMINATIONS

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In November, 1948, an anonymous survey was conducted by the College of American Pathologists[1] to determine the efficiency of clinical laboratories in conducting chemical analytical tests. No doubt the results will do much in improving the accuracy in that the survey, as a follow up, inquired as to the probable causes for inaccuracy. Inasmuch as the chemical determinations were made on identical solutions of glucose, urea, chloride, cholesterol and calcium it should have been possible to obtain similar reports within accepted limits of error. Indeed it should have been much simpler than with the blood samples with which we usually deal.

It is the purpose of this paper to discuss the causes of inaccuracy in blood chemical determinations due to the anticoagulant used in the collection of whole blood. Much has been written and evidently not read on the subject due probably to the inaccessibility of the articles to many. From personal observation I have seen amounts of anticoagulant added to a blood specimen varying from a small pinch up to an eighth of a teaspoonful per 5 to 10 cc. of blood. Also I have noted that solutions are often dried by indefinite temperatures in an oven or on a hot plate. To correct these two fallacies will add much to accuracy.

I. Choice of Anticoagulants for Blood Chemistry

The literature is vast and often confusing on this matter. However the use of oxalates is most generally accepted. Its effect is due to the precipitation of calcium which is essential in the clotting process as insoluble calcium oxalate.[2] The choice of the oxalate depends to a great extent upon whether it will interfere with the determination by adding a foreign substance[3] and whether it will cause a shrinkage of cells where the plasma is to be examined.[4] The amount of anticoagulant should be restricted to that which will not interfere with an analysis, not cause hemolysis or produce an abnormal distribution of water and electrolytes between cells and plasma.[5]

Oxalates

Potassium oxalate is recommended for general use by a large number of writers whose names are shown in Table 1.

• Third Award, ASMT Convention, Boston, Mass., June, 1951.

Table 1

POTASSIUM OXALATE

Folin and Wu[6]	Peters and Van Slyke[10]
Todd and Sanford[7]	Bodansky[3]
Sister M. Alcuin, O.S.B.[3]	Magath and Hurn[11]
Stitt, Clough and Clough[4]	Levinson and MacFate[12]
Hepler[9]	Simmons and Gentzkow[13]
Hawk-Oser-Summerson[5]	Bray[14]

Some investigators prefer sodium oxalate. They claim that potassium oxalate interferes with uric acid determination. These are shown in Table 2.

Table 2

SODIUM OXALATE

Karr, Reinhold and Chornock[15]
Kolmer and Boerner[16]

Others claim that lithium oxalate is preferred especially in uric acid determinations. These are shown in Table 3.

Table 3

LITHIUM OXALATE

Simmons and Gentzkow[13]
Bray[14]
Todd and Sanford[7]

Heparin

Heparin is effective as an anticoagulant but it is not in general usage.[3, 13] It is said to be by far the most satisfactory anticoagulant.[5] Its expense may be the chief prohibitive factor.[3]

Citrate

Citrate is undesirable.[6] It causes cell shrinkage, may interfere with the determination of uric acid and the precipitation of protein.[11]

Defibrination

Defibrination may be desirable for studies of mineral contents and total base of blood.[3, 5, 11]

Sodium Fluoride and Thymol

Sodium fluoride combined with thymol[17,18,19] in the proportion of 10:1 (dry powder) is an excellent anticoagulant and preservative for blood. Sander[17] claims that constant values can be obtained for a period of from five to six days not only for sugars, but for urea (with non-urease methods), uric acid, creatinin and non-protein nitrogen. This is the preferred anticoagulant for the use of general practitioners who must mail blood for chemical analysis to a clinical laboratory.

Sodium Fluoride

Sodium fluoride only is an excellent anticoagulant but has not been found effective as a preservative of blood unless sterile precautions are observed.[19]

II. Amounts of Anticoagulants

Oxalate

The amount of oxalate recommended does not vary greatly. Most authors agree that not more than 3 mg. and that usually 2 mg. per cc. of blood is best. Table 4 gives an outline of these findings.

Table 4

AMOUNT OF OXALATE

Folin and Wu [6]	20 mgs. potassium oxalate per 10 cc. blood.
Sister M. Alcuin [3]	2 drops of 20% potassium oxalate per 10 cc. blood or 20 mgs. per 10 cc. blood.
Todd and Sanford [7]	20 mgs. potassium oxalate per 10 cc. blood or 2 drops 20% solution.
Stitt, Clough and Clough [8]	Not more than 3 mgs. potassium oxalate per 1 cc. blood. .05 cc. of 30% neutral potassium oxalate.
Kolmer and Boerner [16]	0.5 cc. hot saturated solution sodium oxalate.
Bray [14]	20 mgs. potassium oxalate per 10 cc. blood (2 drops of 20% solution) or 10 mgs. lithium oxalate per 5 cc. blood.
Simmons and Gentzkow [13]	1.5 mg. lithium oxalate per cc. blood. 2.0 mgs. potassium oxalate per cc. blood.
Karr, Reinhold and Chornock [15]	30 mgs. sodium oxalate per 15 cc. blood.
Hawk-Oser-Summerson [5]	1 to 2 mgs. potassium, lithium or sodium oxalate per cc. blood.
	0.1 cc. of 10% potassium oxalate per 6 to 10 cc. blood.
Levinson and MacFate [12]	30 mgs. potassium oxalate per 15 cc. blood.
Magath and Hurn [11]	1 cc. of 2% solution potassium or sodium oxalate per 5 to 20 cc. blood.
Peters and Van Slyke [10]	Concentrations of 0.3% and more cause serious alterations in the constituents of blood and even frank hemolysis.
Hepler [9]	Less than 3 mgs. potassium oxalate per cc. blood.

Potassium and Ammonium Oxalate

According to Heller and Paul the study of the distribution of certain inorganic elements in cells and plasma reveals that the cell-plasma ratio varies not only with the nature of the anticoagulant but also with its concentration.[4] The copper sulphate method of Phillips, Van Slyke et al,[20] for the specific gravity of

whole blood and plasma make use of Heller and Paul's observation. Table 5 shows the double oxalate mixture proposed by Heller and Paul and used in the copper sulphate method.

Table 5
AMMONIUM AND POTASSIUM OXALATE MIXTURE

Ammonium oxalate—3 gms.
Potassium oxalate—2 gms.
dissolved in 250 cc. of distilled water to make a 2% solution. Use 0.25 cc. (5 mgs.) per 5 cc. of blood.

Heparin

Table 6 shows the amount of heparin to be used.

Table 6
HEPARIN

Bodansky [3]	1 mg. to 25 to 50 cc. blood.
Simmons and Gentzkow [13]	0.2 mg. per cc. blood.
Hawk-Oser-Summerson [5]	0.2 mg. per cc. blood.

Table 7
SODIUM FLUORIDE AND THYMOL MIXTURE

Sander [17]	10 mg. sodium fluoride and 1 mg. thymol per cc. blood.
John [18]	20 mg. sodium fluoride and thymol mixture (10:1) per 10 cc. blood.
Levinson and MacFate [12]	40 mgs. sodium fluoride and thymol (20:1) per 15 cc. blood.

Table 8
SODIUM FLUORIDE

Roe, Irish and Boyd [19]	10 mg. sodium fluoride per cc. blood.
Hawk-Oser-Summerson [5]	10 mg. sodium fluoride per cc. blood.

III. Effect of Heat on Solutions of Anticoagulants

According to the Handbook of Chemistry and Physics[21] oxalates decompose upon heating. In the directions for the preparation of oxalate tubes for chemical determinations I have observed that some writers do not take this into consideration. They direct that the tubes be placed on a hot plate or in an oven to dry at no designated temperature.[15, 16, 7, 22] Others do give the temperature at which the solution should be dried. These are shown in Table 9.

Table 9
DRYING OF SOLUTIONS OF ANTICOAGULANTS

Hepler [9]	Do not heat over 70 degrees C. or leave the bottom of the bottle or tube in contact with the heat too long as the oxalate is converted to carbonate.
Hawk-Oser-Summerson [5]	Place in an incubator or oven at 100 degrees C.
Simmons and Gentzkow [13]	Dry in an incubator or oven overnight. Do not permit the temperature to exceed 55 degrees C. since at that point and above some of the oxalate may be converted to carbonate.
Stitt, Clough and Clough [8]	Dry without heating in a current of air.
Peters and Van Slyke [10]	Dry without heating in a current of air.
Bray [14]	Dry in the oven under 110 degrees C. High temperatures convert the oxalate into carbonate.
TM-War Dept. [23]	Dry in an incubator or in an oven at a temperature below 80 degrees C., until the water has evaporated. Drying at temperature over 80 degrees C. converts part of oxalate into carbonate and subsequent production of blood clots when tube is used.
Phillips, Van Slyke et al [20]	Dry at temperature not over 40 degrees C.

Practical Points for Consideration

With so many suggestions for the choice of anticoagulants, method of preparation and amount to be used the author has also looked into the literature for other points to be considered as to the advisability of using one or the other. According to Hepler[9] and Bray[14] concentration of potassium oxalate of 3 mg. or more per cc. of blood has the following effects: (1) interferes with the precipitation of proteins (Folin and Wu method); (2) gives too low sugar values (especially with the picric method); (3) frequently causes clouding in Nesslerization. Hawk-Oser-Summerson[5] state that excessive amounts of anticoagulant may interfere with some analyses, may cause hemolysis and may produce an abnormal distribution of water and eletrolytes between cells and plasma. Sodium fluoride and thymol mixture acts as a preservative as well as an anticoagulant and has the advantage of inhibiting glycolytic decomposition of blood sugar, but interferes with urease methods of urea determination.[12,19] Heller and Paul's double oxalate causes little change in red cell volume so it is useful in specific gravity methods but cannot be used in nonprotein nitrogen determinations because of the ammonia content. Potassium oxalate converted to carbonate by heat would not be the proper anticoagulant for recognized chemistry methods.

Potassium oxalate covered with sufficient paraffin oil to overlay the quantity of blood taken is recommended for carbon dioxide content, other gas determinations, pH and plasma chloride determinations. The blood should be collected without a tourniquet if possible. Enter the vein with the needle, remove the tourniquet if used, wait a few moments and fill the syringe. Upon withdrawing the needle place it immediately beneath a layer of paraffin oil in a short wide-mouth bottle containing the oxalate and cautiously add all but one or two cc. of blood. Care must be taken not to mix the blood with the oxalate so vigorously that bubbles form (the same is true for other chemistries).

Before studying the points objecting to potassium oxalate as the anticoagulant for uric acid determinations the author had used it for a number of years and had never had difficulties with the cloudiness which was said to be the interfering reaction. Discarded Petragnani tubes were marked at 5 and 10 cc. capacity and prepared as follows:

1. The tubes for 5 cc. of blood received 0.25 cc. of a fresh 4 percent aqueous solution of potassium oxalate. (10 mgs.)
2. The tubes for 10 cc. of blood received 0.25 cc. of a fresh 8 percent aqueous solution of potassium oxalate. (20 mgs.)
3. The tubes were placed in a slanted position in a wire basket and the oxalate dried at room temperature or in the incubator at 37 degrees C.
4. When used the blood was added to the mark.

As a study in preparation for this paper a series of analyses were made on blood specimens with varying amounts of potassium oxalate to determine whether the amount of potassium oxalate was the cause of the cloudiness in the final cooling in the uric acid test. Non-protein nitrogen and sugar detedminations were also included. Solutions of neutral potassium oxalate up to 30 percent were made and the amount of each to give the amount of potassium oxalate desired was placed in tubes marked at 5 cc. The tubes were dried at room temperature. Other series of tests were made with similar results. Table 10 shows the findings obtained.

Taking the first concentration of potassium oxalate (75 mgs. per 5 cc. blood) in which cloudiness appeared in the uric acid determination and substituting duplicate samples of lithium and sodium oxalate in other blood specimens no cloudiness occurred. Whether cloudiness would take place in the higher concentrations was not determined.

There was excessive bumping and some white cloudiness in the non-protein nitrogen determination at the concentration of

100 mgs. potassium oxalate per 5 cc. of blood. No turbidity was noted due to the Nesslerization. The sugar values increased by six milligrams. The methods used were: (1) Benedict for Uric Acid; (2) Folin-Wu (modified by Simmons and Gentzkow)[13] for Sugar; (3) Folin-Wu for Non-Protein Nitrogen. The determinations were adapted to the Klett-Summerson Photoelectric Colorimeter.

Conclusion

The author in considering the kind of anticoagulant, amount and preparation for blood sample finds the following criteria adequate for accuracy for general use in blood chemistry determinations:

1. Potassium, sodium or lithium oxalate (10 mgs. per 5 cc.) when the blood will be examined immediately. Use 0.25 cc. of a fresh 4 percent aqueous solution for 5 cc. blood (or 0.25 cc. of a higher concentration when more blood is collected—allowing 2 mgs. per cc. blood). Dry at room temperature or in an incubator at 37 degrees C. in a slanted position in a wire basket. Collect blood under paraffin oil for carbon dioxide content, other gases, pH and plasma chloride determinations.
2. Sodium fluoride 10 mgs. and thymol 1 mg. (dry powders) per cc. of blood when the specimen must be sent in the mail.
3. Heller and Paul's potassium and ammonium oxalate mixture for the determination of the specific gravity of whole blood and plasma.

Table 10

Chemical determinations made on same sample of blood with increasing amounts of potassium oxalate solution which had been dried at room temperature.

No.	Potassium Oxalate in 5 cc. Blood	Uric Acid mgms. %	Sugar mgms. %	Non-Protein Nitrogen mgms. %
1.....	10 mgs.	2.78	80	25
2.....	20 mgs.	2.78	80	25
3.....	40 mgs.	2.80	83	25
4.....	50 mgs.	2.80	86	25
5.....	75 mgs.	Cloudy	86	25
6.....	100 mgs.	Cloudy	86	26*
7.....	125 mgs.	Cloudy	86	26*
8.....	150 mgs.	Cloudy	86	26*

* Much bumping occurred and a white precipitate formed. The result given was obtained after centrifuging the specimen.

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A NEW METHOD FOR THE DETECTION OF ALBUMIN IN URINE

A Brief Historical Sketch of the Subject and a Report of 5,000 Comparative Tests*

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The history of the examination of urine and the gradual evolution of the various chemical tests is very interesting. As the time is short, I shall only mention a few of the earlier scientists. Beginning with the classic period of Greek medicine we find Hippocrates (460-370 B.C.) teaching the importance of uroscopy, which is the art of diagnosing disease by the inspection and examination of the urine. If the urine was black, the patient was considered to be in a dangerous condition. If the patient showed signs of frothing at the mouth, convulsions and dim vision, he was considered to be in a critical condition and death was inevitable.

Rufus of Ephesus who lived in the first century and was a friend of St. Paul, was a great physician and wrote a monograph on Diseases of the Kidney and Bladder. This contained many references to the diagnosis and prognostic value of the examination of the urine, saying "the act of examining urine is more important than all other known to medicine." Although he did not recognize albumin as such, he said of tumors of the bladder, "the ulcers . . . do not heal entirely, but soon return, with urine sometimes with sanguinous pus, and sometimes with mucous matters, cloudy with desposits like flour."

Thomas Willis (1694) in England was among the first to place importance on the chemical contents of urine.

Frederik Dekkers (1694) of Leyden first detected albumin in urine by boiling the urine with acetic acid.

Domenico Cotugno (1764) of Italy first demonstrated the presence of albumin in the urine of patients with dropsy.

Richard Bright, in Guys Hospital, London, published his first paper in 1827 and associated albuminuria with kidney disease.

Martin Solon (1838) in Paris is said to have popularized the term albuminuria so that by 1850 it was in common use, meaning kidney disease.

Heller (1841) introduced the nitric acid ring test.

Roberts (1852) modified this test by adding magnesium sulfate to the concentrated nitric acid.

Purdy (1876) modified the heat and acetic acid method by adding sodium chloride solution to the urine before adding the acetic acid.

It is of interest to note that we find in the literature a great

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contrast between the paucity of data on albumin excretion and tests for its determination, as compared with the vast accumulation of data on sugar excretion and tests for its determination.

According to the returns of the questionnaire sent out in 1935 by the American Society of Clinical Laboratory Technicians (the year before we adopted the name of Medical Technologists) to hospitals throughout the country, the most commonly used tests for the detection of albumin in the urine were the heat and acetic acid, nitric acid ring, and sulfosalicylic acid tests. The latter was used least often because it was expensive and the approved method took more time to perform.

From a review of the text books and laboratory manuals recommended by the "Curriculum for Schools of Medical Technology" published by the American Society of Clinical Pathologists, we find that the same three methods are recommended.

From personal communications with a number of technologists and from a review of the books and manuals used by students and technologists today, we find that these three are still the most commonly used methods for albumin determination.

The several tests most commonly used for the detection of albumin in urine are not new. However, there have been several variations and modifications suggested during their years of use.

I shall review briefly the evolution of these three most popular methods in routine clinical analysis.

The first test mentioned, the boiling of the urine with acetic acid, was used for years. It is still a standard and accepted method. Slight variations in technic have been adopted but the basic method was ever the original coagulation or precipitation by heat and acetic acid.

The rationale for using this method was that certain proteins are coagulated by heat and others are precipitated by acid. The old method of making cheese was to let the milk sour and then heat it. This separated the casein from the whey.

In 1841 Heller's nitric acid ring test was introduced. This produced a white zone of precipitated albumin at the point of juncture.

In 1852 Roberts modified Heller's method by adding magnesium sulfate to the concentrated nitric acid. It was thought that this would retard the development of colors at the point of contact.

Purdy (1876) published a modification of the heat and acetic acid method. He added sodium chloride solution to the urine before adding the acetic acid. The addition of sodium chloride was to prevent the interference of mucin by raising the specific gravity of the specimen.

In 1889 Roche was the first to use a 20 per cent solution of sulfosalicylic acid to precipitate albumin in urine.

In 1891 MacWilliams showed the differential value of sulfosalicylic acid using a saturated solution of sulfosalicylic acid for the determination of albumin and globulin.

In 1905 Cammidge published an article about the "pitfalls" of Heller's method and the heat and acetic acid test for the determination of albumin in urine, and admitted that they should be used with care.

In 1913 Kober recommended sulfosalicylic acid as a precipitant for protein in connection with nephelometry.

In 1914 Folin and Denis wrote: "No abnormal urinary constituent is so frequently tested qualitatively as albumin, yet there is, curiously enough, no reasonably accurate and convenient method available for its quantitative determination. The clinical Esbach method is rapid but, like all "sedimentation" methods, it is wholly untrustworthy. And the coagulation methods with accompanying filtrations, washings and weighings, or nitrogen determinations, are so tedious and laborious that they are seldom used. Whether or not quantitative determinations of albumin in urine are capable of yielding much valuable information, suitable methods for the determination of this abnormal urinary constituent are manifestly needed."

Folin described a turbidity method which was a modification of Kober's. Folin was, as far as we know, the first to apply this principle of using sulfosalicylic acid to the determination of albumin in urine. In 1922 Folin simplified the original method by carrying out the precipitation of albumin by sulfosalicylic acid in small graduated test tubes.

Folin's turbidity method was based on the turbidity produced by the precipitating action of sulfosalicylic acid on the albumin in the urine and compared the turbidity so produced with that made under the same conditions with a standard protein solution.

About this time several investigators in Germany published papers recommending the "new method" using the sulfosalicylic acid as the simplest, most accurate, least expensive, and fastest method to use in the determination of albumin in urine.

In 1921 Sanford and Magath wrote a paper demonstrating reasons for the preference of sulfosalicylic acid as a qualitative test for albumin.

In 1921 Exton published the first of several papers on the determination of albumin in urine using sulfosalicylic acid. He popularized the general use of the method suggested by Folin. He simplified the method and suggested that his special reagent was satisfactory for both qualitative and quantitative testing for albumin in urine. His reagent consisted of sodium sulfate and sulfosalicylic acid. He claimed that the sulfosalicylic acid method

was so reliable that it was not necessary to check specimens by other methods.

One of his papers reported tests made on 60,000 specimens showing that urines "loaded to a great excess with uric acid, urates, phosphates, oxalates, urea, creatinine, and other urinary constituents" invariably failed to produce any cloudiness at all with his reagent. A number of pathological urines containing resins, oils or gummy acid, and emulsifying substances, which gave false reactions with Heller's and heat and acetic acid tests, gave consistently negative results with his reagent. Urines which gave misleading reactions with Heller's and the heat test, because of substances which had been added to preserve the specimens, (usually thymol or excessive amounts of formaldehyde) were found to give correct results with the Exton sulfosalicylic acid reagent.

Exton claimed that Heller's nitric acid ring test or any stratification or layer test could not be made to yield reliable quantitative or repeatable results with any technic. With the heat test, it was found that a number of uncontrollable factors, physical and chemical, such as hydrion concentration, kind and amounts of salts in solution, intensity of heat applied, etc., affect the character of the coagula and prevent the achievement of uniform results. Other variables such as the presence of certain drugs and preservatives also detract from the reliability of these tests.

In 1925 Exton concluded that "the specific and quantitative features of the sulfosalicylic acid method imparted an accuracy and uniformity to albumin determinations which made them clinically valuable to a degree hitherto impossible."

In 1925 Kingsbury and Clark published a paper entitled "The Rapid Determination of Albumin in Urine" in which they claimed that an accurate and rapid method was needed for determining albumin in urine. They agreed that the Heller's ring test and the heat and acetic acid test, even under the best conditions, were subject to great variation. The method of reporting as trace, slight trace, one plus, etc., was very rough and varied with the analyst, and the character of the urine. They describe an improved sulfosalicylic acid nephelometric or turbidimetric method with permanent standards.

In reviewing the literature many other methods for the determination of albumin were found. As the scope of the field of chemistry grew, so did the number of chemicals found that precipitated protein. Some of these included citric acid, picric acid, chlor-auric acid, chloro-platinic acid, mercuric chloride, molybdic acid, phosphotungstic acid, silico-tungstic acid, trichloracetic acid and tannic acid.

One text book entitled "Technical Methods for the Tech-

nician," lists twenty-four different methods and reagents. The determination of albumin in urine, like procedures in other fields, has been studied by many and there is always the investigator who thinks his own test is the best. But over the years, the three methods we have presented in detail remained the most popular, namely: heat and acetic acid, nitric acid, and sulfosalicylic acid tests.

In performing all these tests for albumin it is essential that the urine be perfectly clear. If it is not clear, filtration through filter paper or centrifugation will usually suffice for clarification. In case this is ineffective, the urine may be shaken with purified talc, infusorial earth, animal charcoal, kaolin or powdered magnesia. The solution is then filtered. This will remove a part of the albumin by absorption, but the remainder can be detected more easily.

The urine should also be acid to litmus. Sufficient acetic acid may be added to acidulate it.

If bacteria are abundant in an alkaline urine, some of the bacterial proteins may go into solution and respond to tests for albumin.

In extremely concentrated urine, certain of the urinary salts may interfere with the test for albumin. In such cases, dilution of the urine will render the result more definite, even though the concentration of the albumin is thereby reduced.

Mucin, mucoid and nucleo protein which are loosely designated as mucin, are present in normal urine. Increased amounts may be mistaken for albumin in some of the procedures. If the urine is diluted with water and acidified with acetic acid without heating, the presence of a white cloud indicates the presence of mucin. If the urine is acidified and filtered, most of the mucin will be removed and thus exclude this source of error.

Albuminous urine foams markedly on shaking and the foam remains a long time. This gives a rough indication of the presence of albumin before the tests are made.

Normal individuals excrete approximately 0.075 grams of albumin in 24 hours. This amount is too small to be observed my the simple tests in general use for the detection of protein in urine; and there is no reason to want to show its presence in routine chemical examination.

Heat and Acetic Test

The first method used to determine albumin in urine was the heat and acetic acid test. There are two slightly different methods which have been used for years—

1) Heat 5 cc urine to boiling in a test tube. A precipitate is due to albumin or phosphates. Acidify the urine slightly by the

addition drop by drop (3-5 drops) of 2 per cent acetic acid. If the cloudiness is due to phosphates it will disappear. If it is due to albumin it will not only fail to disappear, but will become more flucculent in character, since the reaction of a fluid must be acid to secure the complete precipitation of the albumin by heat coagulation. Certain resins may be precipitated by the acid, but the precipitate due to this may be easily differentiated from the albumin precipitate by reason of its solubility in alcohol.

2) Fill a test tube two-thirds full of urine and gently heat the upper half of the fluid to boiling, being careful that this fluid does not mix with the lower half. A turbidity indicates albumin or phosphates. Acidify the urine slightly by the addition of 3-5 drops of dilute acetic acid, if the turbidity is due to phosphates, it will disappear.

Purdy's Heat Test

The reagent consists of a saturated solution of sodium chloride and 50 per cent solution of acetic acid. The test is performed by filling a test tube two-thirds full of urine, adding one-sixth the volume of saturated solution of sodium chloride and 5 to 10 drops of 50 per cent acetic acid. Mix and boil the upper inch of the mixture on a gas flame, holding the tube with the fingers near the bottom. A white cloud in the heated portion shows the presence of albumin.

The addition of the salt solution raises the specific gravity and prevents the precipitation of mucin.

Heller's Test

About 1 cc of concentrated nitric acid is placed in a small (12 x 100 mm) tube. By means of a pipette having a small rubber bulb at one end and a ragged non-tapering tip at the other end, an equal amount of urine is allowed to flow down the side of the tube without inclining or moving from the rack. By this procedure, a perfect stratification may be obtained with great rapidity. In the presence of albumin, a white zone of precipitated protein will be observed at the point of juncture of the two liquids. If the protein is present in very small amounts, the white zone may not form until the tube has been allowed to stand for several minutes.

Urine containing bile reacts with nitric acid containing a little nitrous acid to give the play of colors referable to the action of nitric acid on bilirubin.

Robert's Test

This test is carried out in the same manner as Heller's test except that Robert's reagent (5 parts saturated magnesium

sulfate and 1 part concentrated nitric acid) is substituted for the nitric acid. Saturated magnesium sulfate contains 76.9 grams per 100 cc water. With this test, colored rings do not form and the test is slightly more sensitive than Heller's but is subject to the same disadvantage in that nucleo protein and mucin are precipitated.

Sulfosalicylic Acid Test

The Exton reagent is prepared by dissolving 200 grams of sodium sulfate in 750 cc of water with the aid of heat. Cool, add 50 grams of sulfosalicylic acid and dilute to 1,000 cc with water. To perform the test, mix equal parts of the urine and reagent in a test tube. A white cloud shows the presence of protein.

Bence Jones protein causes a heavy precipitate which clears on boiling, and will reappear on cooling.

Secondary proteoses may also cause a cloudiness when the mixture cools.

These methods require the use of test tubes, droppers, glass funnels, pipettes, filter paper, conical or centrifuge tubes, various liquid reagents, and the heat of Bunsen burners or alcohol lamps.

New Filter Funnel Method

I now would like to present data on a new method using a sulfosalicylic acid compound.* The principle underlying this new albumin test is acidification and clarification of the urine and the detection of albumin by sulfosalicylic acid in one operation by the utilization of a specially prepared disposable funnel. In order to increase the stability of the test, a form of sulfosalicylic acid which is non-hygroscopic—yet which is as sensitive as ordinary sulfosalicylic acid has been developed.

This special funnel consists of two distinct parts—a filter paper cone and a paper funnel. The cone, which is the inner part, is made up of a special grade of filter paper impregnated with a 25 per cent citric acid solution. It is so constructed that there is sufficient acid to convert 5 cc of an alkaline urine with a pH of about 8 to an acid urine with a pH of about 3.

The paper funnel into which the cone is inserted is coated with a sulfosalicylic acid compound. The double funnel is so constructed that any material that is placed in it will be acidified and clarified in the inner funnel before it comes in contact with the sulfosalicylic acid compound in the outer funnel, which precipitates any albumin present, causing a turbidity to develop in the filtrate. The cloudiness appears immediately.

The funnels are constructed so that any turbidity which re-

* Special filter funnel called ALBUMIN TEST (DENCO)—developed by John Henry Beckley, M.D., Director, Research Laboratory, The Denver Chemical Mfg. Co., Inc., 163 Varick Street, New York, N. Y.

sults in the filtrate is due entirely to the presence of albumin regardless of the condition of the urine prior to it passing through the funnel.

The technic for using the new funnel method is very simple. There is no chance of variations or modifications—

1) Open the funnel by pressing the top gently.
2) Place funnel in test tube.
3) Fill funnel with urine specimen. The capacity of the funnel is 5 cc, but smaller amounts can be used satisfactorily.
4) If albumin is present, a cloudiness or turbidity appears immediately. The amount of the cloudiness is proportional to the amount of albumin present.

Five thousand specimens of urine were examined by well trained technologists.* Comparative tests were made. All tests were done by the new funnel method using the heat and acetic acid as a control method.

A number were also examined by the nitric acid ring test, by Purdy's heat method, and by the sulfosalicylic acid method. In performing the tests, the two or more methods were done independently and the results recorded. The new method checked very closely with the older method, and in many respects it was more advantageous.

A small number of specimens showed a smaller amount of albumin present with the heat and acetic acid method than with the new funnel method. Some of these were checked by other methods and the results agreed with the funnel method. The personal equation of the analyst has a lot to do with variations in technic and the readings. Sometimes duplicate examinations of the same specimen by heat and acetic acid method failed to give the same results. The amount of acid added, the length of time heated, and the time the tubes stood before reading, all had an effect on the report.

Duplicate examination of specimens by the funnel method checked each time. Duplicate specimens examined using Exton's reagent checked also.

The variations of readings between the paper funnel and the standard methods were not marked, i.e. they varied between trace and one plus or a faint trace and a trace, and occasionally between one plus and two plus.

In the course of the work, it has been noted that a number of substances present in pathological urines did not give false positive reactions with the new method. Preservatives such as thymol and formaldehyde, did not produce any false positive reactions.

The presence of alkaline phosphates, urinary salts, mucin,

* Tests were performed under the supervision of Dr. Samuel A. Goldberg, Director of Laboratories, Presbyterian Hospital, Newark, New Jersey.

bile, resins, oil, gummy acids, or deep colors did not affect the reading of the tests.

From the point of view of simplicity of technic and safety of use, this new funnel method is a marked improvement over the commonly used methods which require various test tubes, pipettes, filter paper, glass funnels, Bunsen burners or alcohol lamps and various liquid reagents.

There is no breakage of test tubes using this new method, such as so often happens when the top of even a Pyrex tube is flamed. The test tubes need not be Pyrex. The washing of the tubes is much easier, as by this method the precipitated material does not cling to the glass as it does when heated. There is no odor in the laboratory when this method is used. Due to the fact that the urine was acidified by passing through the first filter funnel, amorphous phosphates and other chemicals that cause a cloudiness with the heat and acetic acid method did not occur.

After comparing the results, we feel that the new funnel method is much more sensitive, simple, accurate, and faster than the other methods. By simple modification, this method may be used as a quantitative method. Standards may be prepared with known amounts of protein and used for comparison.

SUMMARY

A short history of the evolution of tests for albumin in urine is given.

A new method for determining the presence of albumin in urine is described, using a specially prepared filter and paper funnel through which the urine passes and is acidified, clarified, and acted upon by sulfosalicylic acid—all in one operation.

A report is given of 5,000 routine urine specimens examined for albumin by this new method compared with a standard method. The results agree perfectly.

The new method is performed all in one step without previous centrifuging or filtering. No pipettes, reagents or heat are required.

Alkaline urines and those containing mucin, bile, resins and some other substances, which give false positive readings with other methods, did not affect this method.

This new method is specific for the precipitation of proteins.

Results may be reported on a quantitative basis if described by comparing with protein standards.

The precipitation is very quickly completed and results may be read immediately.

This test is sensitive for small amounts of albumin.

The chemicals in the funnel are stable and do not deteriorate with prolonged storage.

The technic is very simple and the personal variations in technic are eliminated.

The new method is very economical as it saves time and glassware, and eliminates the necessity of apparatus and heat.

The results of duplicate tests check perfectly.

It is free from the inaccuracies of layering.

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EDITORIAL

A Word for Youth

Medical Technology belongs to YOUTH. It should be a mature youth, but the interest in something new, the striving toward more satisfactory results, the dissatisfaction with the way "grandfather did it," all prove the point. It is the well-trained newcomer from the progressive hospital school of medical technology who can take hold and perform tests that haven't been heard of by some of the more "experienced" technicians. It is the "youth" in medical technology, in the very make-up of the profession, that makes for recognition of progress in the techniques used, for recognition of that fact that no blood count is "routine."

Youth with maturity—which means that it is not only the very young who qualify, but those who through the years can retain an enthusiasm and devotion to a profession which proves ever fascinating—the young in heart as well as in years—brings to medical technology a significance all its own.

R. M.

On "Values"

"But there is a great difference between organization for economic advantages and organization for professional advancement. The former deals with material things while the latter with ideals and standards of public service. While medical technologists should be concerned with the former for security, their primary interest must be in those things that will win for their profession a larger measure of confidence and respect. The profession of medicine has advanced and given expanding service because of the public recognition that physicians and surgeons have been able to help and relieve the ills of the human body.

"The workers in any profession lift it to higher levels by ideals and patterns of unselfish service rather than by selfish personal requests.

"Let us all work together for a bigger and better American Society of Medical Technologists."

Excerpts from a letter to the Editorial Office from
Mary C. Elliott, MT (ASCP), Johnstown, Pennsylvania.

AMONG THE NEW BOOKS

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY: By W. A. Newman Dorland, A.M., M.D., F.A.C.S., Lieut.-Colonel, M.R.C., U. S. Army; Former Member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association. New, 22nd edition. 1736 pages, with 720 illustrations, including 48 plates. Philadelphia and London: W. B. Saunders Company, 1951. Price $10.00.

The volume no doctor's office nor laboratory can afford to be without, this new edition of the American Illustrated Medical Dictionary is bound in the same familiar cover, but contains all the new terms as well as the old ones. It is as complete a compilation of the terms used in Medicine, Medical Biography, Chemistry, Dentistry, Veterinary Science, and Biology as can be found. The pronunciation, derivation, and definition of terms are clear and concise as in the earlier editions.

AN ATLAS OF HUMAN ANATOMY: By Barry J. Anson. Philadelphia: Saunders, 1950. 518 pages. $11.50.

This volume is made up of excellent illustrations of regional anatomy. Preparation by an anatomist skilled in research, together with clinicians and competent medical artists, results in a volume containing a rather new departure in the type of illustrations of structural variations of the human body. Legends for the illustrations give clearer explanations than the usual material of this type. This makes the volume more valuable for the medical student or technologist whose knowledge of the subject is rudimentary at best. The Atlas, therefore, would be a valuable adjunct to the laboratory library.

CLINICAL DIAGNOSIS BY LABORATORY EXAMINATIONS: By John A. Kolmer, M.D. 2nd edition. New York, Appleton-Century-Crofts, 1949. 1212 pages. Illustrated. Cloth. $12.00.

The new edition of this practical volume has been greatly enlarged by the inclusion of much new text as well as many illustrations. This new material brings it up to date, especially with such information as is current on the laboratory diagnosis of virus diseases. Antibiotic therapy, and Rh-hr factors are other points of current interest.

Although concerned primarily with clinical interpretation, the chapters on laboratory techniques and practical applications of laboratory examinations in this volume are those which have the most appeal for the technologist. To make the library more complete, the material contained herein would prove of value. We can recommend it highly to the school of medical technology as a valuable reference.

THE CARE AND BREEDING OF LABORATORY ANIMALS: Edmond J. Farris, Ed. New York; Wiley; London: Chapman and Hall, 1950. 515 pages. $8.00.

This volume is one that should be in the library of every laboratory wherein animals are used. Its contents are such that we wonder why such a book hadn't been written long ago. Details of feeding, breeding, housing are all given, together with information concerning some of the more common diseases of laboratory animals. For general purpose reference, the material is more than adequate for the usual clinical or public health laboratory although additional references might be necessary from the research angle. In such instances, however, adequate reference material is mentioned.

Fifteen authors contribute to the volume which covers the animals commonly used (mice, rats, guinea pigs, rabbits, hamsters) as well as amphibia, and the certainly less common as the monkey, ferret, domestic fowl, fishes, and opossum.

The subject of breeding and mating habits is well covered and will answer many of the questions of the small-time breeder of laboratory

animals. Housing takes up a considerable portion of the volume and gives many pointers which may be "taken or left," depending upon the school of thought to which one belongs. Feeding of the laboratory animal is touched upon also, but most of the responsibility for that routine is placed upon the commercial house which specializes in that field of work.

Generally speaking, this volume will prove of much value to the laboratory worker whose references in this department have heretofore been almost entirely lacking.

ABSTRACT

Electrically Operated Bunsen-Type Burner, Sol Roy Rosenthal, M.D., Ph.D., Chicago, Ill. Jr. Lab. & Clin. Med., 37:327-330, (Feb.), 1951.

There definitely is need for an electrically operated Bunsen-type burner. In many communities, laboratory procedures must sometimes be omitted for lack of gas, or gas must be installed at great expense for this specific purpose.

This article on the electric burner gives one a good description of the instrument as well as the method of operation, together with some very good pictures illustrating its use in urinalysis, chemistry, and bacteriology, also one showing the mobility of the Bacti-burner.

The heating unit is a lava cylinder operated by a toggle switch in the base. A movable deflector functions to conduct heat radiation away from the operator. Slots are provided for needle holders. For bacteriologic procedures, the bacteriological needle or loop is placed in the cavity of the core when cylinder is glowing and allowed to rest without holding. Contact of loop with glowing lava gives best results. The hazards of working with virulent organisms are reduced by incinerating within a cavity, thus confining spattering within it. In sterilizing test tubes, they are held directly against the outside of the core. Transferring is done in the usual manner. Cotton plugs will ignite if touched to coils. For sterilizing the field of operation, transferring to Petri dishes and so on, the burner may be grasped by the base. For heating fluids in test tubes, roll tube over that portion. A special type of stand is used for heating fluids in beakers, so that the bottom does not rest on the core.

This burner makes clinical laboratory work possible in any room where there is an electrical outlet. It performs practically all functions of the gas-operated type with the advantages of flexibility, ease of operation, reduction of hazards to the operator, absence of "cold" portions of flame, elimination of absence of flame due to drafts and kinking of rubber tubing. It is ideally suited for all gradual heating of fluids.

The burner is manufactured by the Precision Scientific Company, 3737 West Cortland Ave., Chicago, Ill.

Esther Lamont.

Milwaukee, Wis.

THE GAVEL

It would be the nice and expected thing to open this column by saying that it has been a pleasure to serve you this past year. I believe, however, that honesty is the best policy, especially when one is sure to get caught in their dishonesty, and there are too many of you who know the work, the headaches, and the heartaches involved in the office of a national presidency. I can conscientiously say, though, that there have been numerous pleasures in serving you, and it has been very rewarding. Thank you for allowing me the privilege of sharing thus in the progress of our Society.

For the stumbles in the progress of 1950-51, I can but humbly apologize. For the accomplishments of that period, let us all give credit where it is due: the members, as well as the chairman, of the Standing Committees deserve the sincere appreciation of every one of us. The organization is not an 'it'; the organization is 'us'—every one of us. The work of the organization is done by the committees appointed for their specific fields of endeavor. To try to list the persons and their accomplishments would be an impossible task; there is not room on this page, and there are many who have contributed so quietly that we do not hear of it. Therefore, let me say that to every person who has joined in any effort of the Society this past year goes the heart-felt gratitude of all of us.

Equaling my personal humility is my official pride in turning this space to the use of my successor, Miss Lavina B. White, M.T. (ASCP). With the magnificient support which you will give her, she will lead you through the most successful year in our history, 1951-52.

Vernal J. Schene, M.T. (ASCP).

In accepting this token of office from Vernal Schene, it is my sincere hope that the ideals and work of the American Society of Medical Technologists will move along in the same smooth and even tone that makes for progress in our organization. In looking back over the advance made by the society under the former officers, there seems to be an intricate and successful pattern of progress which one does not wish to change or disrupt. It is understandable, however, that one should desire to bring some good contribution to the office and its responsibilities in line with the best interests of the society.

It is here that the coordination of activities may be done, and where the individual or group may cast a burden for understanding and possible solution, or may wish to bring a contribution for the advancement of the profession of medical technology. Let us all work for ASMT, and then ASMT will work for us.

Lavina B. White.

CONVENTION 1951

The Massachusetts Association of Medical Technologists, Inc., and New England were honored and delighted to play hosts to the American Society of Medical Technologists National Convention in June. Your visit was a never-to-be-forgotten happy occasion and a landmark in the annals of MAMT history.

Mrs. Elinor Judd, MT (ASCP),
Public Relation and Publicity Chairman,
MAMT 1951-52.